ESTABLISHMENT OF ECTOMYCORRHIZAL FUNGI ON ROOTS OF BIRCH (*Betula* spp.).

by

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To my parents

DECLARATION

I declare that the work in this thesis is my own, that the thesis has been composed by myself and that none of the material contained herein has been submitted for any other degree or personal qualification.

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SUMMARY

Spatial and temporal successions of ectomycorrhizal fungi on birch trees have been reported, and species involved have been grouped as "early" and "late" stage according to the time of first appearance of their fruitbodies in the temporal sequence.

In an experimental model system, aimed to reproduce these patterns and study mycorrhizal establishment along root systems, small birch (Betula spp.) saplings from a colliery spoil site and with known fingal symbionts were planted into troughs of brown earth from a treeless site with low indigenous levels of mycorrhizal inoculum. The roots were arranged longitudinally along the bases of the troughs to enable precise and repeatable inoculation and observation of the root systems without disturbance of the plants. The inocula, from 6-8 week old liquid cultures, represented species from early, middle and late stages of the observed fruitbody successions (Laccaria proxima, Hebeloma crustuliniforme and Lactarius pubescens respectively) and were applied at various positions along the root systems. After 2.5 years' growth, the patterns of mycorrhizal development on the root systems and the occurrence of fruitbodies in troughs mirrorred, to a degree, the reported spatial succession of fruitbodies on birch, but the relatively short timescale was insufficient for changing patterns of development to be observed.

The effect of soil type on the establishment of mycorrhizal fungi on birch root systems was not examined in the troughs, but was the basis of a series of experiments involving the simplification of microbial populations in a range of soils by microwave irradiation. In this study, the degree of establishment of

mycorrhizas by late-stage fungal species (represented by Lactarius pubescens) tended to be enhanced by previous exposure of the soil to microwaves. As late-stage species are usually poorly infective from dispersed inocula in unsterile conditions, but infect in sterile conditions, the recorded increase in infectivity is at least partly attributable to a reduction in competition from the soil microflora. The experiments also revealed marked differences in the growth of seedlings in response to microwave treatment of different soils.

In a final series of experiments aimed at finding physiological differences between early and late-stage fungi, seedlings and fungi were grown in axenic conditions with the provision of varying amounts of sucrose, nitrogen or phosphorus, or at different osmotic potentials. The main response observed was to sucrose concentration : late-stage species (L.pubescens, Leccinum scabrum and Amanita muscaria) tended to form more mycorrhizas when sucrose was provided at high (1%) than at low (0.01%) concentration. Also, an effect of genotypic variation of B.pubescens was shown when one fungus (Laccaria tortilis) established significantly greater infection of one clone of birch than of another from spores applied to rooted cuttings.

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CHAPTER 1

INTRODUCTION

1.1. General Introduction

The term "mycorrhiza" was first used by Frank (1885) to describe the composite structures that result from associations between roots of forest trees and some soil fungi. These conspicuous structures were characterised by a lack of root hairs and the presence of a fungal sheath, which further study revealed to be hyphal pseudotissue, surrounding the root tips and making them appear short and stubby. Microscopical examination has shown that hyphae enter the root from the sheath but penetrate between, rather than into, the cortical cells so forming the characteristic "Hartig Net" which is visible in cross sections of roots; from this finding was derived the name "ectotrophic" mycorrhizas, or ectomycorrhizas (Frank,1887). Fungi forming ectomycorrhizas with the roots of woody perennials are usually members of the Basidiomycotina or,less often, of the Ascomycotina.

Since these early studies, other associations involving fungi and plant roots have been grouped under the term "mycorrhiza". The most common type, involving possibly 90% of vascular plants, is termed "endotrophic" (Trappe,1977); in this type the roots are morphologically similar to non-mycorrhizal roots (Sanders,Mosse & Tinker,1975) but are infected by hyphae that grow into the cortical cells. These hyphae can branch profusely within root cells to form "arbuscules" (Melin,1923) and can swell to form "vesicles", hence the alternative names "arbuscular" or "vesicular-arbuscular" mycorrhizas. Other types of mycorrhiza include those associated with orchids and those associated with members of the Ericaceae;some of these types exhibit characteristics of both

ecto- and endo-mycorrhizas and are termed "ectendomycorrhizas".The work in this thesis is concerned only with ectomycorrhizas.

1.2. Fungal dependence on the host.

Ectomycorrhizal fungi differ from many other members of the Ascomycotina and Basidiomycotina in that they have generally poor abilities to utilize complex carbon polymers such as are found in wood,leaf litter and humus (Melin,1925). There are some reported exceptions, such as the mycorrhizal fungi *Lactarius deliciosus* (Lindeberg,1948), *Tricholoma fumosum* (Norkrans,1950) and *Xerocomus subtomentosus* (Lundeberg,1970), which can utilise some plant polymers. But in general, the ectomycorrhizal fungi cannot saprophytically grow in normal soil conditions; instead they depend on host-derived carbon sources and are ecologically obligate biotrophs (Lewis,1973). In axenic conditions, however, many species can grow on rich laboratory media.

Movement of carbohydrates from host plants to these fungi was demonstrated clearly by Melin & Nilsson (1957) and Lewis & Harley (1965 a). In the former study $^{14}CO_2$ was supplied in the atmosphere around pine seedlings bearing mycorrhizas, and in the latter study agar blocks containing ^{14}C sucrose were placed on cut stumps of beech; in both cases the ^{14}C label was found to accumulate in the fungal sheath, where it was found in the "fungal carbohydrates" mannitol and trehalose, and in glycogen. These compounds are largely unavailable for use by the plant, and thus a concentration gradient of plant sugars such as sucrose is established, maintaining a flow of carbohydrate to the mycorrhizal fungus (Lewis & Harley,1965 c; Lewis 1975). In essence, therefore, the fungal sheath acts as a sink for plant photosynthates (Shiroya *et al*,1962; Nelson 1964; Lister *et al*,1968; Schweers & Meyer,1970). Other workers (Reid & Woods,1969; Reid,1971; Hacskaylo,1973) have proposed a two-way

system of carbon transfer because ^{14}C has been found in the foliage of pine seedlings adjacent to plants supplied with ^{14}C-glucose or sucrose when the ^{14}C-fed and non-fed seedlings were linked by mycelia of mycorrhizal fungi. Lewis (1973) pointed out that labelled carbon could be taken up into plants as amino compounds, and so two-way transport of carbon could occur by this means rather than in the form of carbohydrates. Nevertheless, recent studies have demonstrated clearly the existence of interplant carbon transfer via fungal bridges, particularly via mycelial strands, and have emphasised the potential of such connections to transfer substantial amounts of nutrients between plants (Read & Finlay,1985; Finlay & Read,1986 a,b).

Björkman (1942) drew attention to the fact that the degree of mycorrhizal infection of a root system is related to the amount of photosynthate reaching the roots. By shading plants and thus decreasing the photosynthetic rate, he was able to reduce the degree of development of mycorrhizas. Binding of the stems of 3-year old pine saplings with thin wire, to prevent translocation of sugars from the needles to the roots, caused inhibition of mycorrhizal formation; when the wires were removed and new conducting vessels developed, then mycorrhizas developed once again. Mycorrhizal development therefore was suggested to depend on the availability of sugars - particularly soluble sugars - in the roots, and Björkman (1942) proposed that unless these sugars were present at sufficiently high levels, mycorrhizal fungi would not infect.

The dependence of mycorrhizal fungi in their hosts is seen conspicuously in the production of fungal fruitbodies. Rommell (1938) severed the roots of spruce trees with iron sheets and observed no fruiting beyond the barriers; he concluded that this was caused by cessation of carbohydrate flow from the host to the mycorrhizal fungus. Later experiments involving decapitation

of pine seedlings, or the removal of their needles or covering of needles with black bags (Hacskaylo,1965) and later the defoliation of birch trees (Last *et al*,1979) showed again that interruption of photosynthesis inhibits fruitbody production, but that fruiting can resume after the foliage regrows or is re-exposed to light. Mycorrhizal fungi in general, therefore, depend on the host plant for their source of carbon, and in this respect they are parasitic. Nevertheless, their presence can confer benefits on the host, as described below.

1.3. Benefits conferred to plants by mycorrhizal fungi.

1.3.1. Increased uptake of nutrients.

The value of mycorrhizal associations to plants was demonstrated by Melin (1917) who observed that pine and spruce seedlings planted on a freshly drained peat bog remained stunted and became chlorotic unless they had been infected with mycorrhizal fungi. Since these early studies it has often been shown that mycorrhizas play a significant role in the mineral nutrition of trees (Hatch,1937; Melin & Nilsson,1950,1953,1955,1958; Harley & Brierly,1954; Melin *et al*,1958) and also in drought tolerance (Cromer,1935; Harley,1940; Goss,1960; Lobnow,1960, Theodorou & Bowen,1970; Uhlig,1972; Griffin,1972; Theodorou,1978) and protection of roots from soil-borne pathogenic fungi (Levisohn,1954; Zak,1964; Marx,1970; Marx & Davey,1969; Sylvia & Sinclair,1983).

The mechanisms of enhanced mineral nutrient uptake by mycorrhizal associations have been intensively studied. Non-mycorrhizal plants are thought to absorb a substantial proportion of their mineral nutrients through root hairs, but when tree roots develop ectomycorrhizas the formation of root hairs is suppressed and their functions are served by hyphae that ramify into the soil from the fungal sheath encasing the root tip. By their branching and extension

these hyphae provide a surface area for absorption many times greater than that of root hairs (Hatch,1937) and hyphal aggregates such as mycelial strands, in particular, have been shown to translocate phosphates over distances exceeding 40 cm (Finlay & Read,1986 b). Mycorrhizal mycelia can also remain active in mineral nutrient uptake for longer than do root hairs (Bowen & Theodorou,1967). Bowen & Rovira (1967) observed that whereas phosphate-uptake by non-mycorrhizal roots of *Pinus radiata* D.Don occurred predominantly near the apex (in the zone of elongation), such uptake could occur along the length of a mycorrhizal root tip.

Hatch (1937) was among the first to show that the shoots of mycorrhizal pine seedlings contain more phosphorus,nitrogen and potassium than do shoots of non-mycorrhizal seedlings, and greater uptake of other elements such as potassium, sodium, rubidium and zinc by mycorrhizal plants has also been demonstrated (Wilson,1957; Harley & Wilson,1959; Skinner *et al*,1972). Most research, however, has focused on phosphate uptake, because this mineral is required in relatively large amounts by plants and its uptake is stimulated more by mycorrhizal infection than is the absorption of other nutrients (McComb, 1938; McComb & Griffith, 1946;). Moreover, in many soils phosphate is not readily available to tree roots, because it occurs in low concentrations in soil and often exists in insoluble, and hence immobile, forms. In the late 19th century it was believed that mycorrhizas were important because they could absorb nutrients, including phosphate, in forms unavailable to non-mycorrhizal trees (Frank,1894) but both mycorrhizas and uninfected roots can obtain phosphates, at least from a range of organic forms (Paterson & Bowen, 1968; Wild & Oke, 1966; Harley, 1966).

Morrison (1957,1962) demonstrated clearly the effect of mycorrhizas on phosphate uptake and plant growth, using seedlings of *Pinus radiata* in pots

and ^{32}P applied at two levels. When phosphate was supplied at high levels, non-mycorrhizal seedlings grew better than those with mycorrhizas but at low levels of phosphate the mycorrhizal plants grew the better. In the low phosphate treatment, ^{32}P moved rapidly into the shoots of non-mycorrhizal plants but then the rate of phosphate supply to shoots decreased and soon became undetectable; the rapid rate could be achieved again by a further application of phosphate. In the mycorrhizal plants, however, the transfer of ^{32}P into the plant shoots occurred at a slower rate but was sustained for 3 weeks, such that more phosphate ultimately accumulated; further addition of phosphate had little effect in these circumstances. Harley & McCready (1952 & 1953) had previously shown that the fungal sheath prevented the plant from absorbing phosphate at the maximum possible rate, unless the concentrations of phosphate were very high, and that after immersion of roots in 4 M $KH_2{}^{32}PO_4$ solution for 7 hours, over 90% of ^{32}P absorbed by beech mycorrhizas remained in the fungal sheath. Crosslett & Loughman (1966) also concluded that inorganic phosphate, as absorbed by mycorrhizas, is incorporated first into metabolic pools in the sheath. Harley (1966) pointed out the ecological significance of the maintenance of high rates of phosphate uptake and storage in the sheath, with subsequent slow release to the plant, particularly in situations where phosphate periodically becomes available, such as during flushes of decomposition of leaves. Harley & McCready (1981) suggested that phosphate is stored in the sheath as polyphosphate, thereby avoiding problems associated with osmotic and buffering effects that would result from the storage of inorganic orthophosphate; polyphosphate has been observed in the sheath of beech mycorrhizas (Chilvers & Harley, 1980).

Whereas phosphate ions can be unavailable to the roots because of insolubility and immobility, ammonium ions can have low mobility relative to demand because of adsorption to soil particles (Harley & Smith, 1983). In the

upper layers of humus and soil, where most mycorrhizas are found, nitrogen is usually most abundant in the form of ammonium compounds which most mycorrhizal fungi can use effectively for growth in pure culture (Melin & Nilsson, 1957) and can take up efficiently in the mycorrhizal state. Melin & Nilsson (1952,1953) showed that ^{15}N from labelled ammonium compounds and glutamine was absorbed by the extramatrical hyphae of mycorrhizas of *Pinus sylvestris* L. and translocated through the fungus to the root, then to the needles; this uptake of ammonium compounds was metabolically dependent and influenced by carbohydrate supply (Carrodus 1966).

As with phosphate uptake, mycorrhizas with associated mycelial strands can exploit large volumes of soil for the uptake of nitrogenous compounds; the movement of ions to mycorrhizas by convection also suggests that mycorrhizas have a substantial water uptake function (Bowen, 1973) which presumably occurs along a gradient of water potential from soil to the transpiring plant (Duddridge *et al*,1980). Duddridge *et al*(1980) have also shown that mature mycelial strands are internally differentiated such that the transport of water and nutrients towards the root in central vessel hyphae of large diameter, and of carbohydrates from the roots in the outer, narrower sheathing hyphae, can occur simultaneously. Mycelial strands of *Suillus bovinus* (Fr.)O.Kuntze have been shown to take up and translocate water to the host over ecologically significant distances of up to 8 cm (Duddridge *et al*,1980).

1.3.2. Increased drought resistance.

Cromer (1935),Harley (1940),Lobnow (1960) and Theodorou & Bowen (1970) have shown that ectomycorrhizal tree seedlings have greater resistance to drought than do non-mycorrhizal plants - or may have faster recovery after limited drought (Goss, 1960) - but the fungal symbionts themselves vary

considerably in their ability to withstand low water potentials in soil and hence to be of benefit to the host in dry conditions (Bowen & Theodorou, 1967; Theodorou, 1978; Griffin, 1972; Uhlig, 1972; Mexal & Reid, 1973). Worley & Hacskaylo (1959) noted that the total percentage of mycorrhizal roots on pine seedlings decreased as drought conditions increased; but this was due to a large reduction in the incidence of a predominant white mycorrhizal type whereas a black mycorrhizal type (possibly of Cenococcum) was not so markedly reduced. *Cenococcum graniforme* Fr. is notable for its high degree of drought tolerance both in culture (Mexal & Reid, 1973) and in association with roots (Reid, 1979; Piggott, 1982). *Pisolithus tinctorius* (Pers)Coker & Couch is also known to survive well on sites subject to drought conditions (Trappe, 1977) and is produced commercially in parts of the U.S.A. to inoculate, and aid in the survival of, containerised seedlings (Marx *et al*,1982). In general, fungi are able to grow in conditions of much lower water availability than do plants; for example, Uhlig (1972, in Harley & Smith, 1983)reported that fungi mycorrhizal with spruce grew at water potentials below the wilting point of spruce. The mycorrhizal association, with its network of hyphae and hyphal aggregates, is thus capable of contributing to the survival of the host plant in conditions of temporary or long term drought.

1.3.3. Protection from pathogens.

There is considerable evidence that the ectomycorrhizal association can protect young roots against attack by pathogenic fungi (Levisohn,1954; Zak,1964). The sheath itself may act as a physical barrier through which a pathogen must penetrate before reaching the root (Marx & Davey,1969; Marx,1970;Marx,1976) but even the presence of the Hartig net may function in a protective role (Marx & Davey,1969; Marx,1970).

There are reports of antibiotic production by mycorrhizal fungi, both in pure culture and on tree roots. Krywolap *et al.* (1964 a,b) extracted an antibacterial antibiotic from *Cenococcum graniforme* on *Pinus strobus* L.. *P.resinosa* Alt. and *Picea abies* L.Karsten.Some other mycorrhizal fungi produce antifungal agents; for example *Leucopaxillus cerealis* var *piceina* (Peck)ined. produces diatretyne nitrile, active against *Phytophthora cinnamomi* Rands., the zoospores of which were immobilised at a concentration of 2 $mgml^{-1}$ (Marx,1969b). Ross & Marx (1972) found that seedlings of *Pinus clausa* were protected against *P.cinnamomi* by *Pisolithus tinctorius*, and Sylvia & Sinclair (1983 a,b,) reported protection of Douglas fir from *Fusarium oxysporum* Schlecht.emend.Snyder & Hansen by the mycorrhizal fungus *Laccaria laccata* (Scop.ex Fr.)Berk. & Br.

Zak (1964) suggested that mycorrhizal fungi protect plants against pathogenic attack by using surplus carbohydrates in the root, thereby reducing the amounts that are released to stimulate pathogens, and also by the maintenance of a protective microbial rhizosphere population.

1.4. Mycorrhizal inoculation.

For some trees, such as pines which depend heavily on mycorrhizas, a prerequisite for the establishment of man-made nurseries, forests or plantations is a sufficient and appropriate population of mycorrhizal fungi in the soil . The population may be low or non-existent for several reasons; for example, the area may not recently, or ever, have supported ectomycorrhizal hosts, or the density of ectomycorrhizal propagules may have been reduced by long agronomic use or treatments to eradicate soil-borne pathogens (Trappe, 1977).

As far back as 1902, Vysotskii (1902) suggested that mycorrhizal inoculation could aid in the afforestation of Russian grasslands and McComb

(1938) and White (1941) also recognised the importance of introducing mycorrhizal fungi in the afforestation of American prairie land. Much research has focused on the problems of establishing plantations of exotic pines in different parts of the world, particularly in tropical regions (Mikola,1980), but selective inoculation programmes require research into the fungal species and forms of inocula most suited to each site and species of tree.

The earliest method of artificial inoculation was the application of soil from sites supporting ectomycorrhizal hosts into nursery soil or container media for seedlings; in East Africa the "Muguga" mixture (May,1953) was used, containing 10 - 20 %, by volume,of top soil from a pine plantation (Marx,1980). Soil inoculum, although usually effective and easy to obtain , is bulky to transport and may introduce pathogens and weeds into nursery soils, particularly where partial soil sterilisation with steam or chemicals has been used to control existing pathogens and weeds. Nevertheless, the practice is still common.

The planting of mycorrhizal among non-mycorrhizal seedlings, and application of freshly excised mycorrhizal root systems to nursery soil, are also effective methods of inoculation, but like the use of soil inoculum, they do not enable selection of the most appropriate fungal symbionts.

Melin (1925) observed that different ectomycorrhizal fungi can affect hosts in different ways. Trappe (1977) found that any one fungus may markedly increase the growth of a given host in one circumstance but not in another, and Marx (1980) found great variability in mycorrhizal formation by twenty-one isolates of *Pisolithus tinctorius*. There is,thus, a growing realisation that it is desirable to select mycorrhizal fungi with required characteristics for use in purposeful inoculation programmes. The application of mycorrhizal inoculum in the form of fruitbodies, spores or sclerotia enables selection of the most suitable species.

Early records of the use of fruitbody inoculum involve the production of "truffle orchards" by Talon in the beginning of the 19th century (Malecon,1938) ; this practice is still used for mycorrhizal inoculation ,but by the addition of chopped fruitbody tissue to soil (Mullette, 1976). Application of this tissue is, essentially, application of spores as sporocarp tissue decomposes quickly. The use of spore inocula, however, is restricted to fungi such as *Pisolithus tinctorius*, the fruiting structures of which are easily recognized and collected and contain large numbers of spores. Spore inoculum can also be transported easily and stored, although storage conditions have not received detailed investigation and spores do not always germinate consistently – particularly after storage (Fries 1977,1978,1979,1983,1984; Fox,1983,1986). Sclerotia, though ideal in terms of longevity , are not so easily collected and have not received much attention as potential mycorrhizal inoculum (Kropp, 1971; Fox,1983). Pure cultures of mycorrhizal fungi would be the preferred source of artificial inoculum in many situations. They take up little space, are easy to handle, especially if entrapped in polymeric gel (LeTacon *et al*,1985), and circumvent the risk of introducing pathogens and pests, but nevertheless pose problems of a different nature. Not all ectomycorrhizal fungi can be cultured, and many grow very slowly. Also there are doubts concerning their survival time in soil (Marx,1980; Lapeyrie & Bruchet,1985). Perhaps the most important limitation, however, to the commercial use of mycelial inocula is in the selection of the most suitable species for different environmental conditions, although mycelia of *Pisolithus tinctorius* have been used successfully in fumigated nursery soil (Marx & Bryan,1975), and species such as *Hebeloma sacchariolens* and *Paxillus involutus* have been used experimentally for inoculation in a range of soils (Deacon *et al*,1983;Last *et al*,1983). In 1987, F.M.Fox and J.W.Deacon (pers.comm.) inoculated 2 million Sitka

spruce seedlings in commercial conditions with mycelia of *Hebeloma* spp. produced in laboratory culture.

It is important that the inoculant species be able to tolerate the conditions of the outplanting site; these may include extremes of temperature or pH or tendency to drought. Some ectomycorrhizal species – notably *Cenococcum graniforme* and *Pisolithus tinctorius* – are reported to be adapted to extreme environments and selection programmes may enable plantations to be established on sites which are otherwise difficult to reafforest (Molina & Trappe, 1982). However, ~~neither *C.graniforme* nor *P.tinctorius*~~ is an uncommon symbiont of trees in Britain, and native fungi with equivalent tolerance of environmental extremes may need to be sought. Ingleby *et al.* (1985) examined the relationship between temperature (and depth) of coal spoil and the occurrence of mycorrhizas of *Paxillus involutus* and *Scleroderma citrinum* Pers. on naturally regenerating birch in a spoil heap in Midlothian. Examinations over the course of one year showed that whereas Scleroderma-type mycorrhizas occurred predominantly in samples from deeper, warmer parts of the spoil (maximum temperature 24°C, 30 cm below the surface), Paxillus-type mycorrhizas were more abundant in the cooler, surface parts of the spoil. Last *et al.* (1983) showed that extremes of soil pH also may influence the success of mycorrhizal inoculation programmes in Britain. Whereas Paxillus-type mycorrhizas ,previously established on seedlings, were almost equally abundant in two types of peat and two types of mineral soil after 2 years' growth of *Betula pendula* Roth., mycorrhizas of *Hebeloma sacchariolens* Quelet persisted and remained dominant on seedlings during 2 years' growth in two mineral soils and in one peat of pH 4.7 but did not survive in a second peat of pH 3.4. Mycorrhizas of *Amanita muscaria* (L.ex Fr.)S.F.Gray, previously established on birch seedlings in these experiments, did not persist when the seedlings were transplanted to any of the four soils. Thus it may be

necessary to select the most suitable mycorrhizal species for each proposed outplanting site, for a successful mycorrhizal inoculation programme. But site factors alone may not account for some of the failures of inoculant mycorrhizal fungi to persist and spread on roots. Rather , there is evidence of natural sequences or successions of mycorrhizal types on tree root systems, and the introduction of a fungus at an inappropriate stage in the succession may result in its failure to colonise roots.

1.5. Successions.

The most detailed study of mycorrhizal successions was begun in 1971, when an experimental plot of birches (*B.pendula* and *B.pubescens* Ehrh.) was established at the Institute of Terrestrial Ecology, Bush Estate. Within 2 years of planting the trees (Last, Mason, Wilson & Deacon, 1983), fruitbodies of *Hebeloma crustuliniforme* (Bull.ex.St.Amans)Quel. and *Laccaria tortilis* (Bolt.)S.F.Gray)Cke. were observed around tree bases; in the following year earth fans of *Thelephora terrestris* (Ehrh.)Fr. were noted and by year 4 there were fruitbodies of *Lactarius pubescens* (Fr.ex.Krombh.) and *Inocybe lanuginella* (Schroed.) Konrad and Maublanc (Last *et al*,1983). By year 6 another nine species had been recorded (Last et al., 1983), including *Leccinum* sp., and in year 10 *Cortinarius* spp. and *Russula* spp. were first observed; by this time, however, fruitbodies of *H.crustuliniforme* and *Laccaria* spp. were seldom seen.

The fruitbodies were not distributed randomly around the trees; rather they tended to occur in rings. Usually when fruitbodies of each species first appeared they did so close to the trunk and in subsequent years rings of progressively increasing radii were observed. Thus the mean distances from the tree trunk of fruitbodies of *H.crustuliniforme* in years 2,4 and 7 were 22,47 and 93 cm respectively, whereas the first appearance of *L.pubescens* in year 4 was at 34

cm and mean distances of 58,120 and 182 cm were recorded in years 7,10 and 12 (Ford, Mason & Pelham, 1980; Last *et al.*,1981,1984b).Unlike those other species, fruitbodies of *Laccaria* spp. tended to occur in clumps, possibly following lines of secondarily thickened roots; nevertheless, again they occurred at progressively increasing distances from the trunk in successive years. These observations on the occurrence and positions of fruitbodies led Ford, Mason & Pelham (1980) to propose that there is both a spatial and a temporal succession of mycorrhizal fungi, detectable as trees age.

Rings of fruitbodies of mycorrhizal fungi, and their outward movement with time , have also been reported by other workers - for pines by Tominaga (1975) and Becker (1956) and for eucalyptus by Gardner & Malajczuk (1985). . Also, characteristic associations have been noted between some fungi and trees of different ages. *Thelephora terrestris*, frequently associated with pioneer colonisers of waste ground (Trappe & Strand,1969), is commonly found in nurseries both in Britain and North America and occurs regularly on seedlings on glasshouse experiments at I.T.E.,Bush (Fleming,1983b;Fox,1983). *H.crustuliniforme* has been observed on nursery seedlings of Douglas fir (Trappe & Strand,1969), *Pinus radiata* (Chu-Chou,1979) and *Pinus* spp. (Lamb,1979), and other *Hebeloma* spp. have been reported on young trees of *Tilia cordata*Mill and *Picea sitchensis* (Bong.)Carr. (Watling,1981). *Laccaria* spp. have been reported as early colonizers of *Quercus* spp. (Watling,1981) and *Eucalyptus* spp. (Chu-Chou & Grace,1982) and in 3-5 year old stands of *Pinus radiata*.

Inocybe spp. have been recorded on *Eucalyptus* spp. only in stands over 6 years old (Chu-Chou & Grace,1982), although they were observed by Fleming *et al.* (1984) on one-year old seedlings of *B.pendula*. *Suillus verrucosum* and *Amanita muscaria* were never found on trees in stands less than 10 years of

age (Chu-Chou & Grace,1982). On Douglas fir, fruitbodies of *A.muscaria* were restricted to trees over 13 years of age (Chu-Chou & Grace,1981) and none has yet appeared on the 16 year old stand of birches at I.T.E.,Bush Estate. In India it was observed that fruitbodies of *A.muscaria* were common on 16 year old *Pinus patula* Schl. and Cham. (the number counted being 310 per tree) but rare on 5 year old trees (3 fruitbodies observed per 100 trees) (Last *et al*,1981).

The sequential appearance of fruitbodies of mycorrhizal fungi in relation to tree age is thus a widespread phenomenon, and not restricted to birch in experimental plots. However, it would be unwise to base evidence of mycorrhizal succession solely on the occurrence of fruitbodies, as demonstrated by Harper & Webster (1964). Single species of coprophilous fungi inoculated into sterilized rabbit pellets took different lengths of time to fruit; these times corresponded to those taken for fruiting in the temporal succession of fungi in fresh pellets in which all the species were initially present. From this Harper & Webster (1964) concluded that each species requires a different minimum period of vegetative growth before a fruitbody is formed, and the observed succession is thus not necessarily indicative of successional vegetative phases. This clearly is a different type of succession from one in which fungal species arrive and find conditions favourable for germination and root infection at different times, these being regulated by nutrient availability, rhizosphere microflora and perhaps other factors. Warcup (cited in Mason *et al*,1982) examined the mycorrhizas below fruitbodies of mycorrhizal fungi around birch and found there to be spatial distribution of mycorrhizas related to fruitbody occurrence. Similarly, Tominaga (1975) found that mycorrhizas of *Tricholoma matsutake* (S.Itoe & Iman)Sing. were abundant below or close to the location of fruitbodies of this species.

Supporting evidence for an age-related succession of mycorrhizal

fungi has come from dissection of soil cores taken at various distances from the birch trees around which fruitbody distributions had been detailed (Deacon,Donaldson & Last,1983).Hebeloma-type mycorrhizas occurred mainly in outer sampling positions, Lactarius-type mycorrhizas occurred in all zones, though they were most abundant mid-way between outer and inner positions, and Leccinum-type mycorrhizas were found in cores taken from positions close to the trees. This study thus provided independent confirmation of spatial successions of the mycorrhizal fungi. However, when seedlings were planted into replicate cores from different positions around the trees they did not neccessarily become mycorrhizal with the dominant mycorrhizal types in the cores; instead unknown types formed in the inner cores and Hebeloma-type mycorrhizas formed in the cores taken 100 cm from the tree. No mycorrhizas of *Lactarius* spp. or *Leccinum* spp. developed on the seedlings, even in cores in which these were the dominant mycorrhizal types as determined by dissection of the cores.

Similarly, inoculation of seedlings in unsterile soils with pure culture mycelial inoculum (Deacon *et al*,1983) or basidiospores (Fox,1983) of *Lactarius* spp. or *Leccinum* spp. was unsuccessful in the establishment of these mycorrhizal types, whereas *Laccaria* spp. or *Hebeloma* spp. readily formed mycorrhizas in equivalent conditions.

As a result of these findings and the order of the observed fruitbody succession it was proposed that mycorrhizal fungi of birch can be divided into two broad categories - "early-stage" and "late-stage" species. Early-stage species can colonise the roots of young seedlings and saplings and so appear early in the temporal succession. Late-stage species are unable to establish infection on the roots of seedlings from dispersed inocula in unsterile conditions and appear in the succession only when the trees are more mature. In aseptic

conditions, however, with only the plant and mycorrhizal fungus present, both early and late-stage species readily formed mycorrhizas (Mason *et al*,1983a).

In further studies of successional patterns,Fleming (1985) showed that seedlings planted into undisturbed soil around birch trees readily became mycorrhizal with *Lactarius pubescens*, but if planted into soil which had been cored the seedlings became infected with other, usually early-stage, species of mycorrhizal fungi. This suggested that late stage species can establish mycorrhizal infection on seedlings if the inoculum is attached to a food base - the host tree - but severance of this connection results in no mycorrhizas of late stage species being formed. This seems to be a major difference between early- and late-stage species;essentially, the former group are able to establish infection on seedlings in unsterile conditions from a range of inoculum sources whereas late-stage species need to be attached to a considerable food base in order to do so. More recent work (Fleming *et al*,1986), however, has suggested that in old natural woodland soils or in sites that have borne a full-term crop of an artificially established stand of trees, mycorrhizas of *Lactarius* spp. can develop readily on seedling roots even in isolated (cored) samples of soil. It thus seems that the distinction between early- and late-stage mycorrhizal fungi (or at least the placement of *Lactarius* spp. within such a scheme) is influenced by soil properties associated with length of afforestation (Fleming *et al*,1986).

It has been suggested that species such as *Laccaria* and *Hebeloma* are pioneer fungi, colonising newly planted woodlands and open land invaded by sapling trees (Watling,1981), consistent with the attributes of "r" strategy (McArthur & Wilson, 1967).

Organisms following r-strategy use energy for fast growth episodes accompanied by high reproductive effort in the exploitation of potentially

productive habitats. Early-stage fungi, such as *Laccaria* spp., seem to fit this description in that they generally are easy to culture, they produce numerous small fruitbodies , even on seedlings in pots, and are able rapidly to colonise young seedlings in competition with soil microorganisms.

Late-stage species tend to follow "K" strategy as defined by McArthur & Wilson (1967) by being poorly competitive, for example for seedling roots, by producing fewer but larger fruitbodies (Deacon *et al.*,1983) and by forming extensive networks of hyphal aggregates - mycelial strands - in soil around tree roots; examples cited by Deacon *et al.* (1983) include *Lactarius* spp. and *Leccinum* spp.

Grime (1979) suggested that most basidiomycetes, which would include ectomycorrhizal species, fit the pattern of the K-strategist. This may be true of decomposer basidiomycetes but within ectomycorrhizal fungi there seem clearly to be members which adopt relatively more of an r-strategy.

1.6. Mechanisms of succession.

Despite much evidence of successional patterns there has been little work on the mechanisms of succession of mycorrhizal fungi. At least four general mechanisms, as outlined below, might operate to account for the observed successions of mycorrhizal fungi on birch.

1).There may be a sequence of arrival of fungal species. Although this mechanism cannot be discounted from the fruitbody mapping studies at I.T.E. Bush, the seedling-based experiments of Fleming (1983,a,b),Fox(1983) and Deacon *et al.* (1983) have indicated that there are some species of mycorrhizal fungi - the late-stage species - that would not have infected the young birch in

the experimental plot at Bush even if inocula of these fungi had been present at the outset. Perhaps the time required for fruiting by each species varied, as described for the succession of coprophilous fungi by Harper & Webster (1964), thus giving a temporal succession of fruiting. Indeed the fruitbodies of late-stage species such as *Lactarius* spp.,*Leccinum* spp. and *Amanita* spp. tend to be large and to occur in low numbers, whereas those of early-stage fungi such as *Hebeloma* spp.,*Inocybe* spp. and *Laccaria* spp. are generally smaller and thus may require less mycelial resources for their production.Even if this generalisation holds, still the evidence of Deacon *et al.* (1983) demonstrated that the distribution of fruitbodies was matched by distribution of mycorrhizas in the soil, so the observed spatial succession was not simply related to fruitbody occurrence.

2).Successions may reflect changes in the host-symbiont relationship with age of trees or age of individual regions of the root system. As a tree grows its potential for photosynthesis increases and the availability of carbohydrates to mycorrhizal fungal associates also may increase. If some mycorrhizal species have a particularly high requirement for sugars then these species may not be able to form mycorrhizal associations in the early years of tree growth. In this respect it is notable that a wide range of mycorrhizal species will form mycorrhizas with seedlings in axenic conditions, but such studies have usually involved supporting media of high sugar concentration [e.g. 1% glucose as used by Mason(1980)]. Meyer (1966) analysed the sugar content of different regions of roots of 2 year old beech seedlings with abundant mycorrhizas and recorded a clear sugar concentration gradient within the root system. The highest sugar content was in the oldest, upper part of the root system. By this or some other physiological means, there may be some differential "receptivity" to infection by different mycorrhizal species along the root system.

3).A gradient of sugar concentration within the roots may also result in variation in the composition of the rhizosphere microorganisms in different parts of the root system, depending on the composition of the root exudates. Smith (1969) found that the root exudates of 55 year old maple differed considerably from those of seedlings in that the exudates of the older trees contained a wider range of sugars and a greater proportion of organic acids. Variation in the nature of exudates may occur even between older and younger regions of the root system of one tree and may directly influence the composition of the rhizosphere populations in these regions, which in turn may produce substances that can affect root permeability (Norman, 1955,1961) or root metabolism. An alteration in the rhizosphere microflora may also enable or inhibit the growth of mycorrhizal species in different regions of the root system and the mycorrhizal species themselves may regulate the succession patterns by interacting on the root systems. Frankland (1981) described how two fungal species may colonise root surfaces very close together, perhaps growing over each other or meeting at their colony margins; replacement could follow when the growth of one fungus is inhibited and the other grows over or through it. The replacement process may be passive, such as when one species simply grows faster than the other, gains access to nutrient supplies and hence prevents further growth of the other, or it may be active, such as in hyphal interference as observed by Ikeduigwu (1976,a,b,) and Ikeduigwu & Webster (1970) for coprophilous species. There has been little research into interactions between mycorrhizal fungi but this may be an important factor in the mechanism of succession.

4).Mycorrhizal successions may reflect changes in soil physical or chemical properties as trees age or as the soil around an individual tree is explored to a greater of lesser extent by roots. There will be numerous such

changes - both temporal and spatial - around an individual tree or beneath a population of trees, and some of these changes will result from differential deposition of organic matter such as leaf litter or even differential availability of water (Douglass,1960;Last *et al*,1983) penetrating the tree canopy.

1.7. Aims and objectives of the work in this thesis.

The work carried out in this thesis was designed to investigate some of the factors involved in the distinction between "early-" and "late-stage" mycorrhizal species as have been defined by Deacon *et al.*(1983), and hence to investigate factors that might help to explain successions of mycorrhizas on birch at I.T.E., Bush Estate. Specifically, three main aspects were studied, as follows.

(1). A range of reported early- and late-stage mycorrhizal fungi were compared with regard to their tolerance of different water potentials and their abilities to form mycorrhizas with birch seedling roots in axenic culture when the basal medium contained different levels of carbohydrates and mineral nutrients. In addition, competitive interactions between the mycorrhizal fungi were examined in culture and in the presence of plant roots.

(2).The effect of the soil microflora on the efficacy of mycorrhizal development from naturally occurring soil inocula or introduced inocula was examined, by exposure of soil to microwave irradiation which was designed selectively to inhibit some components of either the natural mycorrhizal flora or the general soil population of microorganisms.

(3).The development of different mycorrhizal fungi was studied on differently aged parts of birch tree root systems in a specially designed model system in a glasshouse, where it was hoped that a degree of uniformity of soil conditions could be maintained along the length of the root systems.

CHAPTER 2

METHODS AND MATERIALS.

2.1. Materials

2.1.1. Fungal isolates

Isolates of ectomycorrhizal fungi used in this study were obtained from fruitbody tissue and were maintained as actively growing colonies on half-strength Potato-Dextrose Agar and on Modified Melin-Norkrans Agar. Their provenances are shown in Table 2.1.1.

2.1.2. Growth media

Various agar media were used for the maintenance of fungal isolates and for some pure culture studies. Those supplied commercially are as follows:

- Davis agar (Gelatine Ltd.,Leamington Spa,U.K.)
  (used 10 gl^{-1})
- Malt extract agar (Oxoid Ltd.)
- Potato dextrose agar (Oxoid Ltd.)
  (used mainly half-strength with Davis agar added to restore the concentration of agar to 10 gl^{-1}).
- Tryptone-soy agar (Oxoid Ltd.)
  (used one-quarter strength with Davis agar added to restore the concentration of agar to 10 gl^{-1}).
- Czapex-Dox agar (Oxoid Ltd.)

In addition, the following growth media were prepared as required.

(1).Modified Melin-Norkrans Agar (MMN) (Marx, 1969a).

	gl^{-1}
$CaCl_2$	0.05
NaCl	0.025
KH_2PO_4	0.5
$(NH_4)H_2PO_4$	0.25
$MgSO_4.7H_2O$	0.15
Thiamine HCl	100ug
Glucose	10.0
Davis agar	10.0

This medium was also used, without agar, for liquid culture of isolates.

(1b.)MMN with one-tenth nitrogen concentration.

This medium was prepared as for standard MMN above but with the following modifications:

	gl^{-1}
$(NH_4)H_2PO_4$	0.025g
Na_2HPO_4	0.24g

(1c.)MMN with one-tenth phosphorus concentration.

This medium was prepared as for standard MMN but with the following modifications:

	gl^{-1}
KH_2PO_4	0.075g
$(NH_4)H_2PO_4$	0g
$(NH_4)_2HC_6H_5O_7$	0.212g
KCl	0.187g

(2).Potato Dextrose Medium

	gl^{-1}
Potato extract (Difco)	2.0
Glucose	10.0

For a solid medium, 10.0g Davis Agar was added.

All media were autoclaved at 121°C for 15 min prior to use.

Table 2.1.1
Origin of fungal isolates used experimentally ; all isolates were obtained from birch on Bush Estate.

Fungus	Isolate code	Date of isolation	Origin *
Laccaria laccata	1	1985	a
Laccaria proxima	19	1983	b
	22	1983	b
Hebeloma crustuliniforme	4	1983	b
	8	1984	a
	13	1984	a
Hebeloma sacchariolens	4	1982	b
	6	1983	a
Hebeloma mesophaeum	1	1985	a
Hebeloma subsaponaceum	2	1984	a
	3	1984	a
Hebeloma spp.	3	1984	a
Lactarius pubescens	4	1982	b
	6	1984	a
Leccinum scabrum	1	1984	a
Amanita muscaria	5	1983	a
	7	1983	a
Tricholoma fulvum	2	1982	b
	4	1982	b

* a = author ; b = L.V.Fleming

2.2. General methods

2.2.1. Germination of birch seed.

Birch seed (*Betula pendula*) was obtained from the Forestry Commision, Alice Holt, in 1984 and aseptically germinated birch seedlings were produced by the method of Pelham & Mason (1978). First, the papery "wings" of the seeds were removed by gentle rubbing between the palms of the hands, and then viable seed was separated from non-viable seed by immersion in Industrial Methylated Spirit.The non-viable seeds floated on the surface of the I.M.S. and were discarded; the remainder of the seeds were removed and dried thoroughly on aluminium foil. Seeds were then sterilised by shaking in McCartney bottles containing 7 ml of hydrogen peroxide solution (30% w/v in water) for 30 min. The seed was then transferred aseptically to plates of water agar (10g Davis agar per litre of water) which were sealed with cling film and incubated in aluminium foil-lined cabinets with continuous lighting from three "Gro-Lux" fluorescent strip lights (40W) in a room maintained at 23°C. Most viable seeds germinated within 14 days.

2.2.2. Production of inoculum of mycorrhizal fungi.

Isolates of mycorrhizal fungi were cultured on agar, in vermiculite-peat or in MMN medium.

For the production of vermiculite-peat inocula,horticultural vermiculite was shaken through a 2 mm mesh B.S. sieve to remove the fine dust; the fraction retained by the sieve was mixed with finely ground peat (pH 3.5) in the proportion of 9:1 (v/v). Batches of 250 ml of this mixture were added to 500 ml Erlenmeyer flasks and moistened with 180 ml MMN solution. The flasks were

plugged with cotton wool and capped with aluminium foil prior to autoclaving at 121°C for 15 min. When cool, the flasks were inoculated with agar discs cut from the margins of actively growing colonies on PDA or MMN agar, with approximately 50 ml of previously colonised vermiculite-peat or with approximately 10 ml of mycelial suspension from previously inoculated liquid culture.

For the production of liquid culture inocula,aliquots of 200 ml MMN solution were added to 500 ml Erlenmeyer flasks which were plugged, capped, autoclaved and inoculated as in the production of vermiculite- peat cultures.

Inoculated flasks were incubated at 20°C for 2-3 months or until the vegetative mycelium had sufficiently colonised the medium.

2.2.3. Propagation of plants from cuttings.

In July 1985, cuttings were taken from 11-year old birch trees (*B.pubescens*) of two clones (9.3D and 9.3G). The cuttings, consisting entirely of that season's growth, were each 5-8 inches long (or longer if available) and were cut from the ends of small branches with secateurs. Cuttings from each tree were placed in labelled polythene bags to conserve moisture during transportation to the propagator-glasshouse.

The glasshouse was equipped with thermostatically-controlled automatic vents, shading as required and spray-mist taps which sprayed the propagator beds every 3 minutes. The cuttings were prepared by cutting the base into a "heel" across a leaf node, dipping this heel into a hormone rooting powder (Strike) containing a fungicide and removing several leaves to reduce moisture loss. They were then planted closely together in labelled rows in an

asbestos-lined peat-grit bed (1:1 v/v) which was approximately 6 inches deep.

After 2-3 weeks the cuttings were tested for rooting; resistance to a gentle tug on each cutting indicated that rooting had taken place and the cuttings could then be excavated carefully and potted into 75 mm diameter plastic pots containing a peat:loam:grit mixture (4:1:1 v/v). For 3 weeks the potted cuttings remained on the mist-bench then they were transferred to another heated glasshouse for "hardening-off", being watered only as required. After a further 2 weeks the cuttings were suitable for experimental use.

2.2.4. Production of mycorrhizal seedlings

Seedlings bearing mycorrhizas of selected mycorrhizal species were produced individually in axenic conditions in boiling tubes (as described in the relevant section) and in unsterile conditions in glasshouse conditions by an adaptation of the method of Mason *et al*(1983a). Plastic seed trays (222 x 165 x 57 mm), without drainage holes, were packed with approximately 150 grey polystyrene tubes (each 75 mm long, 13 mm internal diameter and split on one side)(Telcon Plastics Ltd.). The tubes were packed one-third full with autoclaved and cooled vermiculite-peat mixture moistened with water. The remainder of the tube was then filled with either vermiculite-peat inoculum or with alternate additions of liquid-culture inoculum and sterile vermiculite-peat. Into each tube was carefully planted one aseptically germinated birch seedling and a final layer of sterile vermiculite-peat was packed around the seedling stem. A fitting propagator lid with holes in the top was placed over the tray and the system was transferred to an unheated glasshouse (in summer) or a heated glasshouse with supplementary lighting (in winter). The tubes were watered and shaded as required and the lids were removed after 4 weeks.Within 12 weeks mycorrhizas

were visible on the seedling root systems. Non-mycorrhizal seedlings also were produced in this way by planting the seedlings into sterile vermiculite- peat alone and taking care to prevent contamination by air-borne fungal spores by keeping the propagator lid in position.

2.3. Plant growth conditions.

Plants were incubated in a heated or unheated glasshouse, in growth cabinets or in foil-lined open cabinets, the details of which are provided in the method sections of individual experiments.

2.4. Method of assessment of roots.

Plants to be sampled were brought into the laboratory and the shoot was cut off at the junction with the soil. Growth parameters of the shoot (height and dry weight) were recorded where appropriate. The roots were washed free of soil , sometimes after several hours of soaking in tap water in order to loosen the soil on the roots, under gently running tap water and the roots were placed in a Petri dish for examination.

Numbers of root tips of different mycorrhizal types, dead mycorrhizas and uninfected root tips were counted under a stereoscopic microscope (x6 - x50 magnification). Frequent checks on the identity the different mycorrhizal types were made under a compound microscope by reference to preserved material of different mycorrhizal types.

The distribution of mycorrhizas along a root system was assessed by laying the entire root system on a sheet of glass with an underlying linear scale. In some experiments all root tips were counted; in others the root systems were

cut into 1 cm sections and every third section was examined.

In some experiments the entire root system was then oven dried (at 80°C) and weighed.

2.5. Statistical analysis.

In general, results were subjected to analysis of variance, Student's t-test and the Chi-square test where appropriate, using statistical packages on a GENSTAT program.For analysis of variance the results were transformed to log(x + 1) or angles,to normalise them.

CHAPTER 3

EFFECTS OF MICROWAVE IRRADIATION OF SOIL ON ESTABLISHMENT OF MYCORRHIZAS.

3.1. Introduction.

There have been numerous attempts to alter the composition of the soil microflora, mainly in order to reduce the populations of particular pathogens in the soil and hence enable increased plant growth. Both chemical and physical methods of partial soil sterilisation have been used, such as chloroform (Jenkinson, 1976; Jenkinson & Powlson, 1976; Powlson & Jenkinson, 1976), methyl bromide (Ladd *et al.*,1976; Powlson & Jenkinson, 1976), steam (Baker, 1970), soil heating by solarisation (Katan, 1980; Pullman *et al.*,1981) and, recently, microwave irradiation (Baker & Fuller, 1969; Diprose *et al.*, 1978; Vela & Wu, 1979; Wainwright *et al.*,1980; Ferriss, 1984).

The action of chemicals on soil microflora has provided insight into the effects of partial soil sterilisation, both immediate and long term. It has long been known that compounds that are harmful to plants when in direct contact (e.g. carbolic acid) can stimulate plant growth if applied to the soil before planting. The effect was not, at first, thought to be related to bacterial metabolism but Starkey (1955), Martin (1963) and ZoBell (1964) have shown that almost all organic compounds can be utilised by some microorganisms or group of microorganisms. That the plant growth response following fumigation was directly related to the action of the surviving soil microflora was shown by Altman & Tsue (1965). The initial effect of fumigation by chemical or physical means is the death or injury of non spore-forming organisms (Altman, 1965)

followed by a flush of decomposition as the dead cells are broken down (Jenkinson & Powlson, 1976; Jenkinson, Powlson & Wedderburn, 1976; Powlson & Jenkinson, 1976) and resulting in an increase in the population of the surviving organisms. The action of fumigation in the eradication of pathogens is thus the prevention of regrowth of the detrimental organisms by enabling their replacement in the rhizosphere with increased numbers of less harmful organisms.

There is an increasing move towards a reduction in the use of chemicals in disease control and of the physical methods of fumigation, microwave irradiation of soil has received recent attention.

Microwave treatment of soil is essentially a thermal effect and as such relies on the soil being moist (Baker & Fuller, 1969; Vela & Wu, 1979; Ferriss, 1984). Exposure of soil to 65°C for 30 minutes in soil solarisation tests killed most important plant pathogens and weeds (Pullman *et al.*, 1981) but only 150 seconds of microwave irradiation of 1 kg soil eliminated *Pythium* spp., *Fusarium* spp. and most nematodes (Ferriss, 1984); even 20 second exposure had a marked effect on the viable count of soil microorganisms (Wainwright *et al.*, 1980). Although partial sterilisation of large volumes of soil by microwave irradiation is not yet economically feasible, the technique is fast, simple and efficient in the reduction of populations of microorganisms in soil. Fungi tend to be more susceptible to microwaving than bacteria (Diprose *et al.*, 1978), particularly spore-forming bacteria. The pesticidal activity is direct, there are no long-lasting side effects (such as may be seen in the persistence of some chemicals) and there appears to be less release of nutrients and disturbance to the soil structure and composition than with autoclaving or fumigation with chemicals (Ferriss, 1984).

The experiments in this chapter were designed to investigate the effect of microwave irradiation of soil on plant growth and the survival and infectivity of naturally occurring mycorrhizal inocula in various soil types, and on the establishment of mycorrhizas from added inoculum in the presence of a microwave-altered soil microflora.

3.2. General method.

Soils were collected from field sites and stored, if neccessary, in sealed polythene bags at 4° C until required. They were then seived through a 2mm mesh and their moisture contents were determined by air-drying weighed samples spread on to sheets of aluminium foil. The saturation capacity of the soil was then determined by the perforated box method of Keen & Raczkowski (1921) and the percentage saturation (on a wet weight basis) of the main batch of soil could thus be calculated.

The seived soil was mixed thoroughly and weighed into screw-cap polypropylene tubes (Sterilin Ltd.) of c.10cm height, 3cm diameter and 50ml capacity, each with a small drilled hole in the base for drainage. The weight of added soil was calculated such that the tubes would be filled to within 0.5cm of the rim. The tubes were then randomised and subjected individually to microwave irradiation for fixed lengths of time, with their caps slightly loosened. The microwave oven (Philips Cooktronic 8915) was set at power level '8' for all exposures. After exposure to microwaves, each tube was immediately removed from the oven, its cap was screwed tightly and it was placed on the laboratory bench to cool. Exposure times varied between experiments, based on the findings of previous experiments and based also on knowledge of the temperatures reached by water-filled tubes (Table 3.2.1). Also, in each

Table 3.2.1
Temperatures of water and soils after microwave irradiation for various times.(°C)

Microwave exposure (sec)	Water	Farfield	Bush	Bush	N.grange	Struan	London
0	26	26	26	26	26	26	26
15	-	46.7 ±2.6	-	-	-	-	-
20	-	-	49.0 ±3.1	-	67.7 ±6.4	57.7 ±1.7	70.0 ±2.5
30	44.0 ±1.0	56.3 ±2.7	-	-	-	-	-
40	-	-	-	-	91.3 ±0.7	93.0 ±0.5	86.0 ±1.5
50	-	79.3 ±1.2	-	-	-	-	-
60	81.2 ±1.2	-	82.0 ±0.8	83.0 ±3.8	-	-	-
80	94.0 ±0.6	95.0	-	97.7	>95	>95	>95
180	-	-	95.7 ±0.3	-	-	-	-

experiment a preliminary test was done to record the temperatures of soils immediately after different lengths of exposure to microwaves, the values (Table 3.2.1) being usually means of three replicate tubes which were not used for the main part of the experiment.

Once the soil had cooled to room temperature, one tube from each treatment was reserved for analysis of microbial populations as described later. The remaining tubes were distributed randomly between sub-treatments as appropriate. The main type of sub-treatment involved supplementation of soil with inoculum of a mycorrhizal fungus. In order to achieve this, the soil in individual tubes was emptied into a sterilised tray and a known quantity of inoculum in either vermiculite-peat culture or liquid culture (Section 2.2.2) was mixed thoroughly into the soil before this was returned to the tube, aseptic technique being observed throughout. The control (unsupplemented) tubes were also tipped out and the soil redistributed back into the tubes, but there was no addition of inoculum. In one experiment, a small amount (1.0g) of freshly collected, sieved agricultural soil was added as a supplement to some tubes in a similar manner to that above.

Each tube was wrapped in black plastic to exclude light from the root zone, the cap was removed and the tube was then planted with a non-mycorrhizal birch seedling, 4 - 6 weeks old, raised from surface-sterilised seed on agar plates (Section 2.2.1).

All of these procedures were done within 3h of irradiation. A shallow layer of acid-washed perlite was added to the surface of the soil in each tube, around the seedling, in order to prevent "puddling" or "capping" of the soil during watering. The tubes were distributed between blocks, each containing one replicate of each treatment, fully randomised within the blocks and stood in

propagator trays containing washed, autoclaved grit for support.

The trays were placed in growth cabinets (Fisons model Fi-troton 600H) with light (6.4 klux) supplied by fluorescent tubes in an 18h light: 6h dark cycle and temperature of 18°C (day) and 15°C (night). The tubes were watered with autoclaved distilled water, twice daily or as neccessary, by means of a wash- bottle, care being taken to avoid cross-contamination. Initially it was possible to standardise the watering, but as seedlings grew to different degrees in the different treatments, so the amount of watering was varied according to the needs of individual seedlings.

Experiments were incubated for 12 wk, which was found to be sufficient for the development of mycorrhizas and the detection of treatment differences. On sampling, each tube was soaked for several hours in tap water, in order to loosen the soil; then the soil ball was carefully removed from the tube and the roots were washed under slowly running tap water.

Shoot length was recorded for each seedling, then the shoot was removed, oven dried for 7 days at 80°C and weighed. The roots were arranged linearly on a grid and 1 cm sections were cut with a razor blade from every 3 cm of root length. All root tips in the pooled 1 cm sections of each root system and those attributable to different mycorrhizal types were counted, the types being identified as far as possible from reference material. The dry weight of the entire root system was determined after oven-drying for 7 days at 80°C.

All results were subjected to analysis of variance after transformation to angles or $\log(x + 1)$ as appropriate.

Assessment of microbial populations

From one tube in each microwave exposure treatment, 1.0g of soil was aseptically removed immediately after microwaving and used to obtain a dilution series in sterile tap water down to 10^{-6} (g/v). Aliquots (0.1 ml) of appropriate dilutions were spread on to each of three replicate plates of Tryptic-Soy Agar (TSA) and three of Czapek-Dox Agar plus streptomycin (CDSA). The plates were incubated at 25°C for 3-14 days. Bacterial colonies of different types were sub-cultured for subsequent typing. Fungal colonies (on CDSA) were examined after 10 days' incubation and identified to genus level. In some experiments the populations of fungi were calculated based on colony counts, but in most cases a record was made only of the relative frequencies of different fungal types.

3.3. Experiment 1, with soil from FARFIELD, near Bush Estate.

3.3.1. Introduction.

In this experiment, soil was collected from the top 10 cm from Farfield, an experimental site on which spaced birch trees had been established 11 years previously. The soil, a brown earth of pH 5.1–5.3, was collected fresh from the field and sieved < 2 mm, mixed thoroughly and weighed into polypropylene tubes each containing 43 g fresh weight of soil. The tubes were exposed to microwave irradiation for 0,15,30,50 and 80 sec and there were 14 replicates per treatment. Then soil in half of the tubes in each microwave treatment was supplemented with 1.2 - 2 g vermiculite-peat inoculum of *Lactarius pubescens* from a 6-week old culture; the soil in each tube was removed into a sterilised tray, the inoculum was mixed into the soil thoroughly and then the soil was re-packed into the tube. Four-week old aseptically germinated birch seedlings (*B.pubescens*) were planted, one per tube, and the tubes were arranged in randomised blocks in a propagator base and incubated for 12 weeks in a growth cabinet (18°C day/15°C night) with light supplied at 6.4 klux in an 18 light/6 dark cycle. Results for parameters of seedling growth and mycorrhizal development are shown in Tables 3.3.1 to 3.3.10.

3.3.2. Results.

Seedling height.

Seedling height was significantly affected by microwave irradiation of soils, averaged over the inoculation treatments, but not by supplementation with inoculum of *Lactarius pubescens*, again averaged over microwave treatments

(Table 3.3.1). The pooled results for supplementation treatments showed that seedling height was significantly greater in soils treated for 30,50 and 80 sec, than for 0 and 15 sec, However, microwave irradiation beyond 30 sec exposure did not lead to a further significant effect on seedling height. Also , there was no significant effect on seedling height of microwave irradiation for 15 sec compared with that in untreated soil. Inspection of Table 3.3.1 shows that these responses occurred in both uninoculated and inoculated soils.

Root and shoot dry weights

The results for root and shoot dry weights broadly reflect those for seedling height described above, in that there was a significant plant growth response to irradiation of soil but not to soil supplementation with *L.pubescens*. Again, averaged over the inoculation treatments, a significant growth enhancement occurred following 30 sec exposure to microwaves, but inspection of Tables 3.3.2 and 3.3.3 shows that inoculated and uninoculated treatments behaved somewhat differently in this respect; the major growth responses in unsupplemented soil occurred only after 50 sec exposure whereas they occurred after 30 sec exposure for soil subsequently supplemented with *L.pubescens*. Evidently, dry weights provided a more sensitive assessment of plant growth responses than did seedling shoot heights.

Root : shoot ratio

Root:shoot ratios, calculated on a dry weight basis, differed relatively little between the treatments, with no obvious trend (Table 3.3.4). There was an anomalously high value of 0.9 :1 in soil exposed to microwaves for 30 sec and

Tables 3.3.1 - 3.3.6. Growth parameters of birch seedlings grown for 12 wk in Farfield soil exposed to microwave irradiation for different times and then either supplemented or not supplemented with inoculum of *Lactarius pubescens*; means of seven replicates (means of $\log_{10}(x+1)$ in parenthesis).

Table 3.3.1
Seedling height (mm)

Inoculation	Exposure(sec)						
	0	15	30	50	80	Row	
None	56	51	65	79	84	(1.81)	
+ Lp4	50	45	67	69	75	(1.76)	SED(0.04)
							5%LSD -
Column Means	(1.70)	(1.68)	(1.82)	(1.87)	(1.89)		

SED (0.06)
5%LSD (0.11)
1%LSD (0.15)

Table 3.3.2
Shoot dry weight (mg)

Inoculation	Exposure (sec)					Row	
	0	15	30	50	80	Mean	
None	66.1	59.3	77.1	167.1	183.1	(1.95)	
+ Lp4	44.7	42.2	119.0	145.3	145.7	(1.87)	SED(0.06)
							5%LSD -
Column Means	(1.62)	(1.63)	(1.93)	(2.12)	(2.15)		

SED (0.11)
5%LSD (0.21)
1%LSD (0.27)

Table 3.3.3
Root dry weight (mg)

Inoculation	Exposure (sec)					Row mean	
	0	15	30	50	80		
None	42.8	45.2	51.8	115.1	127.5	(1.78)	
+ Lp4	27.2	31.3	116.8	93.4	106.6	(1.72)	SED(0.08)
							5%LSD -
Column mean	(1.41)	(1.52)	(1.78)	(2.04)	(2.01)		

SED (0.11)
5%LSD (0.25)
1%LSD (0.34)

Table 3.3.4
Ratio of root : shoot dry weights

Inoculation	Exposure (sec)					Row mean	
	0	15	30	50	80		
None	0.6	0.7	0.6	0.6	0.6	0·62	
+ Lp4	0.6	0.7	0.9	0.6	0.7	0·7	SED 1·3
							5%LSD —
Column means	0·6	0·7	0·75	0·6	0·65		

SED 0·1
5%LSD —

Table 3.3.5

Mean number of root tips per seedling

Inoculation	Exposure (sec) 0	15	30	50	80	mean	
None	228.3	310.7	204.3	371.0	394.1	(2.389)	
+ Lp4	149.7	180.6	363.4	395.6	343.3	(2.351)	SED(0.07) 5%LSD -
Column means	(2.134)	(2.30)	(2.368)	(2.524)	(2.523)		

SED (0.115)
5%LSD (0.229)
1%LSD (0.305)

Table 3.3.6

Ratio of number of root tips : root dry weight

Inoculation	Exposure(sec) 0	15	30	50	80	Row Mean	
None	8.7	9.5	3.3	4.2	4.3	6.0	
+ Lp4	5.2	7.8	6.5	2.9	3.4	5.1	SED 1.3 5%LSD -
Column Means	7.0	8.7	4.9	3.6	3.8		

SED 2.1
5%LSD 4.2

then supplemented with *L.pubescens*; the reason for this is unclear, but the effect was consistent in the results for individual replicate seedlings (data not presented). Nevertheless, analysis of root:shoot ratios for 30 sec exposure times for supplemented and unsupplemented treatments using Student t-test showed no significant difference between treatments.

Numbers of root tips

The mean number of root tips per seedling was variable in the experiment as a whole but tended to increase with increasing exposure of soil to microwaves, a statistically significant increase over that in untreated soil occurring after exposures of 30 sec or more (Table 3.3.5). This result again broadly reflects the growth responses of seedling as a whole, but a comparison of numbers of root tips with root dry weight (expressed as a ratio) reveals an interesting pattern. There was a tendency for the number of root tips per unit of root dry weight to decrease with increases in microwave exposure time (Table 3.3.6), demonstrating that proportionally more of the root biomass was allocated to structural components rather than nutrient absorbing components in the larger root systems (of the larger plants) than in the smaller ones. A consequence of this is that proportionally fewer root tips must have been available for infection by mycorrhizal fungi in the larger than in the smaller root systems, on a root dry weight basis.

Mycorrhizal development

Effects of treatments on mycorrhizal development were assessed by recording for each seedling the number of all mycorrhizal root tips as a

percentage of the total number of root tips, and also the numbers of mycorrhizal tips attributable to different fungi expressed as a percentage of the total number of mycorrhizal tips on each seedling (Tables 3.3.7 to 3.3.11). Six main trends were observed, as follows.

1) Overall, there was no significant effect of either exposure time or soil supplementation on the percentage of root tips that became mycorrhizal, although this percentage ranged from approximately 44 to 70 % across the treatments (Table 3.3.7).

2) In the absence of *Lactarius* inoculum, three mycorrhizal types developed, presumably from naturally occurring inoculum in the soil. Only one of these mycorrhizal types could be identified, belonging to *Thelephora terrestris*; but the other types were distinctive, one being typical of an ascomycete and the other being similar to the "Y"-type in the reference material at I.T.E., Bush Estate. The Y-type predominated on root systems in the experiment as a whole.

3) The proportions of different mycorrhizal types were significantly affected by exposure to microwave irradiation as explained in (6) below.

4) *L.pubescens* established mycorrhizal infection only from added inoculum and did so in all treatments, but with a tendency for more infection by *L.pubescens* at the higher exposure times. The differences in this respect between individual exposure times were sometimes statistically significant (Table 3.3.8).

5) The presence of *L.pubescens* significantly reduced the incidence of two of the three other mycorrhizal types, namely Y-type and the ascomycete-type, as explained in (6) below.

Tables 3.3.7 - 3.3.11
Mycorrhizal development on birch seedlings grown for 12 wk in Farfield soil exposed to microwave irradiation for various times and then either supplemented or not supplemented with inoculum of *Lactarius pubescens*; means of 7 replicates (means of angular transformation in parenthesis).

Table 3.3.7
Percentage of root tips with mycorrhizas

Inoculation	Exposure (sec) 0	15	30	50	80	Row means	
None	70.5	57.1	56.8	49.2	53.2	(49.3)	
+ Lp4	44.1	50.5	59.9	51.5	55.3	(48.2)	SED(2.56) 5%LSD -
Column means	(49.1)	(47.8)	(48.6)	(46.4)	(51.9)		

SED (4.05)
5%LSD -

Table 3.3.8
Percentage of mycorrhizal root tips formed by Lactarius

Inoculation	Exposure (sec) 0	15	30	50	80	Row means	
None	0	0	0	0	0	(0.0)	
+ Lp4	39.0	21.9	65.3	53.8	71.1	(44.1)	SED(4.0) 5%LSD -
Column means	(39.2)	(23.8)	(55.0)	(43.8)	(58.9)		

SED (6.33)
5%LSD (12.66)
1%LSD (16.84)

Table 3.3.9
Percentage of mycorrhizal root tips attributable to Y-type

Inoculation	Exposure (sec) 0	15	30	50	80	Row means	
None	78.6	85.3	100.0	75.8	25.9	(65.0)	
+ Lp4	54.7	67.6	23.1	26.5	24.1	(36.7)	SED(5.38)
							5%LSD -
Column means	(63.3)	(66.4)	(59.5)	(38.7)	(26.3)		

SED (8.51)
5%LSD (17.02)
1%LSD (22.64)

Table 3.3.10
Percentage of mycorrhizal root tips attributable to Ascomycete - type

Inoculation	Exposure (sec) 0	15	30	50	80	Row means	
None	0	2.5	0	20.9	45.7	(13.1)	
+ Lp4	0	1.2	0	6.3	0	(1.3)	SED(3.75)
							5%LSD(7.50)
Column means	(0.0)	(3.9)	(0.0)	(16.5)	(15.6)		1%LSD(9.98)

SED (5.92)
5%LSD (11.84)
1%LSD (15.75)

Table 3.3.11
Percentage of mycorrhizal root tips attributable to Thelephora

Inoculation	Exposure (sec) 0	15	30	50	80	Row means	
None	21.4	12.2	0	3.2	28.3	(12.6)	
+ Lp4	6.3	9.2	11.6	13.4	4.8	(13.2)	SED(5.08)
							5%LSD -
Column means	(8.2)	(11.7)	(6.5)	(15.8)	(22.5)		

SED (8.03)
5%LSD -

6) There was, in general, a reduction in the occurrence of Y-tyoe mycorrhizas with long irradiation time in both uninoculated and Lactarius - supplemented treatments, the effect being highly significant overall (Table 3.3.9). The most marked reduction in Y-type mycorrhizas occurred only after 80 sec exposure in the unsupplemented soil but after 30 sec in the soil supplemented with *L.pubescens*. This last observation coincided with a marked increase in mycorrhizal development by Lactarius in soil irradiated for 30 sec (Table 3.3.8), so it seems that the adverse effect of irradiation *per se* on the Y-type fungus was exacerbated by Lactarius-type in conditions in which *Lactarius* could infect well. It is also notable that a marked plant growth response occurred after only 30 sec exposure of soil supplemented with *L.pubescens* compared with after longer exposures (50 sec) in unsupplemented soil (Tables 3.3.1 and 3.3.2). But comparison of Table 3.3.9 with Tables 3.3.1 or 3.3.2 shows that the plant growth response in unsupplemented soil was not closely related to the degree of infection by the Y-type fungus.

In contrast to Y-type, the incidence of mycorrhizas of the ascomycete was significantly enhanced by long irradiation times - indeed, this type of mycorrhiza was present at very low levels or even absent from soils exposed for short times, and present as the predominant mycorrhizal type only at the highest exposure times (Table 3.3.10). As in the case of Y-type, however, the presence of inoculum of *L.pubescens* significantly decreased (P= 0.01) the development of ascomycete-type mycorrhizas and in some instances suppressed this development completely. The greatest degree of development of ascomycete-type mycorrhizas in unsupplemented soil coincided with significant plant growth responses to soil irradiation (at the 50 and 80 sec exposure times). In one of the tubes of soil exposed to 80 sec irradiation, a buff-coloured apothecium, 7 mm in diameter, developed on the soil surface; the seedling in this

tube had a very high proportion of the ascomycete-type mycorrhizas. Unfortunately, the apothecium was removed for identification before it had produced mature spores, which rendered precise identification impossible. Nevertheless, the presence of this fruitbody supports the view that the mycorrhizas had been formed by a member of the Ascomycotina.

Mycorrhizas of *Thelephora* showed a very variable pattern of development in the experiment as a whole, with no significant difference between irradiation treatments or soil supplementation treatments. Inspection of Table 3.3.11, however, suggests that there was an increase in the number of Thelephora-type mycorrhizas in Lactarius-supplemented soil at high compared with low exposure times, but analysis of variance of the data for Lactarius-supplemented soil alone showed there to be no significant difference between exposure times.

3.3.3. Summary.

Increasing microwave irradiation of soil resulted in overall larger seedlings, in terms of height, root and shoot dry weights and total number of root tips, but did not alter the degree to which the root systems became mycorrhizal. *Lactarius pubescens* established infection whenever inoculum was added but not otherwise. Of the three other mycorrhizal types that developed from natural inoculum, Y-type tended to decrease with both increased exposure to microwave irradiation and supplementation with inoculum of *L.pubescens*, whereas the ascomycete-type tended to increase with increasing exposure but was barely evident in the presence of *L.pubescens*. The occurrence of Thelephora was largely unaffected by either exposure to microwaves or supplementation with *L.pubescens*.

3.4. Experiment 2, with soil from BUSH Estate.

3.4.1. Introduction.

In this experiment, soil was collected from the top 10 cm of an experimental plot at I.T.E., Bush Estate on which spaced birch trees had been established 15 years previously. Soil collected fresh from the field was seived < 2mm, mixed thoroughly and weighed into polypropylene tubes each containing 50 - 51 g fresh weight of soil. The tubes were exposed to microwave irradiation (Section 3.2) for 0,20,60 and 180 sec, with 14 replicates per treatment. Then soil in half of the tubes in each microwave treatment was supplemented with 0.1 g of liquid inoculum of *Amanita muscaria* isolate 5 from a 6-week old culture. Aseptically germinated birch seedlings were planted, one per tube and the tubes were incubated in a growth cabinet as for the previous (Farfield soil) experiment. Results for parameters of seedling growth and mycorrhizal development are shown in Tables 3.4.1 to 3.4.10.

3.4.2. Results.

Seedling height

Seedling height was significantly affected by microwave irradiation of soils, averaged over the inoculation treatments, and also by supplementation of soils with inoculum of *Amanita muscaria*, averaged over microwave treatments (Table 3.4.1). The pooled results for supplementation treatments showed that seedling height was significantly greater in soils microwaved for 60 and 180 sec than in untreated soil and in that treated for 20 sec. However, inspection of Table 3.4.1 shows that whereas there was a significant growth response to 60

Tables 3.4.1 - 3.4.6
Growth parameters of birch seedlings grown for 12 wk in Bush soil exposed to microwave irradiation for different times and then either supplemented or not supplemented with inoculum of *Amanita muscaria*; means of 7 replicates (means of log(x+1) in parenthesis).

Table 3.4.1
Seedling height (mm)

Inoculation	Exposure (sec) 0	20	60	180	Row means	
None	51	35	103	44	(1.69)	
+ Am5	47	45	99	97	(1.79)	SED (0.045)
						5%LSD(0.090)
Column means	(1.64)	(1.56)	(1.97)	(1.78)		

SED (0.063)
5%LSD (0.127)
1%LSD (0.327)

Table 3.4.2
Shoot dry weight (mg)

Inoculation	Exposure (sec) 0	20	60	180	Row means	
None	82.7	26.6	222.5	40.6	(1.68)	
+ Am5	48.1	56.6	203.6	237.9	(1.90)	SED (0.087)
						5%LSD(0.174)
Column means	(1.63)	(1.42)	(2.23)	(1.89)		

SED (0.123)
5%LSD (0.246)
1%LSD (0.327)

Table 3.4.3
Root dry weight (mg)

Inoculation	Exposure (sec) 0	20	60	180	Row mean	
None	69.8	22.5	161.5	37.7	(1.62)	
+ Am5	31.8	48.7	159.1	192.6	(1.77)	SED (0.085)
						5%LSD -
Column means	(1.52)	(1.33)	(2.10)	(1.83)		

SED (0.120)
5%LSD (0.240)
1%LSD (0.319)

Table 3.4.4
Ratio of root : shoot dry weights

Inoculation	Exposure (sec) 0	20	60	180	Row means	
None	0.8	0.8	0.7	0.9	0·82-	
+ Am5	0.6	0.8	0.7	0.8	0·92	SED 0·7
						5%LSD -
Column means	0·7	0·8	0·7	0·85		

SED 0·1
5%LSD —

Table 3.4.5
Mean number of root tips per seedling

Inoculation	Exposure (sec) 0	20	60	180	Row mean	
None	172.3	65.7	320.1	100.7	(2.00)	
+ Am5	155.9	139.7	135.1	384.6	(2.23)	SED (0.073)
						5%LSD(0.146)
						1%LSD(0.194)
Column means	(2.07)	(1.83)	(2.37)	(2.20)		

SED (0.103)
5%LSD (0.206)
1%LSD (0.275)

Table 3.4.6
Ratio of number of root tips per seedling : root dry weight

Inoculation	Exposure (sec) 0	20	60	180	Row mean	
None	3.9	3.4	2.0	2.6	2·97	
+ Am5	5.2	4.7	2.2	3.0	3·77	SED 1·8
						5%LSD –
Column means	4·55	4·05	2·1	2·8		

SED 0·6
5%LSD 1·3
1%LSD 1·45

sec exposure in treatments both with and without supplementation, the growth response to 180 sec occurred only in Amanita-supplemented soil. The reason for this is unclear but nine of the ten replicate seedlings in unsupplemented soil exposed for 180 sec were much smaller than might have been expected. There was no significant effect on seedling height of microwave irradiation for 20 sec compared with in untreated soil. This was true for both supplemented and unsupplemented soils (Table 3.4.1).

Shoot and root dry weights

The results for shoot dry weights (Table 3.4.2) broadly reflect those for seedling heights, described above, in that there were significant plant growth responses to irradiation of soil and to supplementation with *A.muscaria*, but anomalously low shoot dry weights in unsupplemented soil exposed to microwaves for 180 sec. The results for root dry weight (Table 3.4.3) followed a similar pattern, except that pooled results for microwave treatments showed no significant effect of supplementation with *Amanita*. Again, averaged over the inoculation treatments a significant growth enhancement occurred following 60 sec exposure to microwaves, and inspection of Tables 3.4.2 and 3.4.3 show that, apart from the 180 sec exposure, inoculated and uninoculated treatments behaved similarly.

Root:shoot ratio

Root:shoot ratios, calculated on a dry weight basis, differed relatively little between the treatments, with no obvious trend (Table 3.4.4). The root:shoot ratios in this experiment were, on the whole, greater than those in the "Farfield"

experiment, averaging 0.87 compared with 0.81 in Farfield soil.

Numbers of root tips

The mean number of root tips per seedling (Table 3.4.5) was variable in the experiment as a whole but tended to increase with increasing exposure of soil to microwaves; again the result for unsupplemented soil exposed to microwaves for 180 sec was low. The results broadly reflect the growth responses of seedlings as a whole. A comparison of number of root tips with root dry weights (Table 3.4.6) showed no significant effect of supplementation with *Amanita* averaged for microwave treatments, but a significant decrease with increasing exposure when averaged for supplementation treatments. Inspection of Table 3.4.6 shows that this decreasing trend was true for both supplemented and unsupplemented soils.

Mycorrhizal development

Effects of treatments were assessed as described in the Farfield experiment (Section 3.3.1). Six main trends were observed as follows:

1) Overall, there was no significant effect of either exposure or soil supplementation on the percentage of root tips that became mycorrhizal, though this percentage ranged from approximately 50 to 65% across the treatments (Table 3.4.7).

2)In both the presence and absence of Amanita inoculum, seven mycorrhizal types developed, presumably from naturally occurring inoculum in the soil. Only three of these mycorrhizal types , belonging to *Thelephora*

Tables 3.4.7 - 3.4.10
Mycorrhizal development on birch seedlings grown for 12 wk in Bush soil exposed to microwave irradiation for various times and then either supplemented or not supplemented with inoculum of *Amanita muscaria*; means of 7 replicates (means of angular transformation in parenthesis).

Table 3.4.7
Percentage of root tips with mycorrhizas

Inoculation	Exposure (sec) 0	20	60	180	Row mean	
None	53.9	52.0	50.3	65.2	(48.0)	
+ Am5	59.3	60.3	51.5	62.3	(50.2)	SED (2.68)
						5%LSD -
Column Means	(50.0)	(49.9)	(46.5)	(49.9)		

SED (3.79)
5%LSD -

Table 3.4.8
Percentage of mycorrhizal root tips attributable to Thelephora

Inoculation	Exposure (sec) 0	20	60	180	Row mean	
None	34.0	18.8	34.9	78.9	(33.7)	
+ Am5	11.5	14.8	18.6	96.7	(33.8)	SED (4.87)
						5%LSD -
Column means	(18.0)	(20.7)	(22.3)	(74.1)		

SED (6.89)
5%LSD (13.78)
1%LSD (18.33)

Table 3.4.9
Percentage of mycorrhizal root tips attributable to H-type

Inoculaton	Exposure (sec) 0	20	60	180	Row mean	
None	39.4	25.1	26.3	16.5	(30.9)	
+ Am5	28.6	20.7	46.5	0.3	(27.9)	SED (5.00)
						5%LSD -
Column means	(36.7)	(34.6)	(37.4)	(9.5)		

SED	(7.07)
5%LSD	(14.14)
1%LSD	(18.81)

Table 3.4.10
Percentage of mycorrhizal root tips attributable to Hebeloma

Inoculation	Exposure (sec) 0	20	60	180	Row mean	
None	19.6	40.6	30.0	4.6	(21.4)	
+ Am5	38.2	50.3	26.9	0.7	(19.4)	SED (4.42)
						5%LSD -
Column means	(21.9)	(25.2)	(28.2)	(6.3)		

SED	(6.25)
5%LSD	(12.50)
1%LSD	(16.63)

terrestris, *Hebeloma* and *Cenococcum*, could be identified, but the other four types were distinctive, two being typical of ascomycetes, one being similar to the "T-type" in the reference material at I.T.E., Bush Estate, and the other termed "H-type". Mycorrhizas of *Thelephora*, H-type and *Hebeloma* predominated on root systems in the experiment as a whole; the other four types occurred too infrequently to be amenable to statistical analysis but none of them was observed in soils subjected to 180 sec exposure to microwaves.

3)The proportions of mycorrhizal types were significantly affected by exposure to microwave irradiation as explained in (6) below.

4)*Amanita muscaria* did not establish mycorrhizas from added inoculum except on one seedling in the 180 sec exposure treatment; nor did *Amanita* establish infection from naturally occurring inoculum.

5)The presence of *Amanita* inoculum did not significantly affect the incidence of other mycorrhizal types.

6) There was a significant reduction in the occurrence of H-type and Hebeloma-type mycorrhizas with long (180 sec) exposure time in both unsupplemented and Amanita-supplemented treatments. In contrast, there was an equally significant increase in the occcurrence of mycorrhizas attributable to *Thelephora* with long exposure times in both soils, coinciding with the reduction of H-type and Hebeloma-type mycorrhizas.

3.4.3. Summary.

Increasing exposure of soil to microwave irradiation resulted in generally larger seedlings, in terms of height, root and shoot dry weights and total number of root tips, but did not alter the degree to which the root systems

became mycorrhizal. *Amanita muscaria* did not establish infection from added inoculum (except on one seedling) or from any naturally occurring inoculum. The addition of inoculum of *A.muscaria* had no significant effect on the development of any of the mycorrhizal types observed. Of the three main mycorrhizal types, both Hebeloma-type and H-type tended to be unaffected by exposure to 20 or 60 sec irradiation but were significantly decreased by exposure for 180 seconds. In contrast, *Thelephora*, although tending to be unaffected by exposure to 20 or 60 sec irradiation, was significantly increased by exposure for 180 seconds.

3.5. Experiment 3 - Reseeding of BUSH soil.

3.5.1. Introduction.

In this experiment soil was collected from I.T.E., Bush Estate as described in Section 3.4.1. and stored in polyethelene bags at 4 ° C for 2 weeks, then sieved < 2mm, mixed thoroughly and weighed into polypropylene tubes each containing 53.5 - 54.0 g fresh weight of soil. The tubes were exposed to microwave irradiation for 0,60 and 180 sec, with 24 replicates per treatment. Each tube was then supplemented with 1.0 g vermiculite-peat inoculum of *Lactarius pubescens* from a 6-week old culture, but half of the tubes in each microwave treatment was further supplemented with 1.0 g of sieved agricultural soil from a field that had borne a wheat crop and was sited several kilometres from any major woodland. In addition,seven polypropylene tubes were filled with 53.5 - 54.0 g of this agricultural soil alone. Aseptically germinated birch seedlings were planted, one per tube, and the tubes were incubated in a growth cabinet as for the Farfield experiment. Results for parameters of seedling growth and mycorrhizal development are shown in Tables 3.5.1 - 3.5.12.

3.5.2. Results.

Seedling height

Seedling height was significantly affected by microwave irradiation of soils, averaged over the inoculation treatments, and by supplementation with 2% agricultural soil, averaged over microwave treatments (Table 3.5.1). The pooled results for supplementation treatments showed that seedling height was significantly greater in soils treated for 60 and 180 sec than in untreated soil, but

Tables 3.5.1 - 3.5.6
Growth parameters of birch seedlings grown for 12 wk in Bush soil exposed to microwave irradiation for different times and then either supplemented or not supplemented with agricultural soil; means of 12 replicates (means of log(x+1) transformation in parenthesis).

Table 3.5.1
Shoot height (mm)

Supplementation	Exposure (sec) 0	60	180	Row means	
None	73	96	79	(1.91)	
+ soil	62	76	82	(1.85)	SED (0.026)
					5%LSD(0.052)
Column means	(1.82)	(1.92)	(1.90)		

SED (0.032)
5%LSD (0.063)
1%LSD (0.084)

Table 3.5.2
Shoot dry weight (g)

Supplementation	Exposure (sec) 0	60	180	means	
None	0.077	0.169	0.110	(0.067)	
+ soil	0.057	0.107	0.119	(0.038)	SED (0.011)
					5%LSD(0.021)
					1%LSD(0.028)
Column means	(0.044)	(0.058)	(0.056)		

SED (0.013)
5%LSD -

Table 3.5.3
Root dry weight (g)

Supplementation	Exposure (sec) 0	60	180	Row means	
None	0.105	0.186	0.127	(0.055)	
+ soil	0.062	0.140	0.108	(0.041)	SED (0.009)
					5%LSD -
Column means	(0.040)	(0.061)	(0.047)		

SED (0.010)
5%LSD (0.021)

Table 3.5.4
Ratio of root : shoot dry weight

Supplementation	Exposure (sec) 0	60	180	Row means	
None	0.7	0.9	0.8	0·8	
+ soil	0.9	0.7	1.1	0·93	SED 0·2
					5%LSD —
Column means	0·8	0·8	0·95		

SED 0·3
5%LSD —

Table 3.5.5
Mean number of root tips per seedling

Supplementation	Exposure (sec) 0	60	180	Row means	
None	129.6	196.6	194.4	(2.192)	
+ soil	83.8	151.9	148.7	(2.033)	SED (0.055)
					5%LSD(0.110)
					1%LSD(0.146)
Column means	(1.98)	(2.16)	(2.20)		
		SED	(0.067)		
		5%LSD	(0.134)		
		1%LSD	(0.179)		

Table 3.5.6
Ratio of number of root tips per seedling : root dry weight

Supplementation	Exposure (sec) 0	60	180	Row means	
None	1.6	2.0	1.7	1·78	
+ soil	2.0	2.1	1.6	2·85	SED 0·21
					5%LSD —
Column means	1·8	2·05	1·65		
		SED	0·33		
		5%LSD	—		

there was no significant difference between seedling heights in soils exposed for 60 and 180 sec respectively. Inspection of Table 3.5.1 shows, however, that the responses to microwave treatment were somewhat different in the presence or absence of the soil supplement. In particular, there was a progressive increase in seedling height with longer exposure time in soil-supplemented soil, whereas the greatest seedling response in unsupplemented soil occurred after 60 sec exposure and there was a diminished effect of exposure to 180 sec in this treatment. This paralleled the response seen previously in Bush soil (Section 3.4).The pooled results for microwave treatments showed that seedling height was significantly reduced when Bush soil was supplemented with fresh agricultural soil.

Shoot and root dry weight

The results for shoot dry weight, pooled for exposure times, reflect the data for seedling height, in that shoot dry weight was significantly reduced by supplementation with fresh agricultural soil (Table 3.5.2). However, there was no significant effect of exposure to microwave irradiation on shoot dry weight, which was surprising in view of the significant effect on seedling height.

In contrast, root dry weight was significantly influenced by exposure of soil to microwaves, but was unaffected by the addition of fresh agricultural soil (Table 3.5.3). Inspection of this table shows that root dry weight was significantly increased in soil exposed for 60 sec as opposed to untreated soil, whereas there was a lesser (and non- significant) response to 180 sec exposure. The results for both supplemented and unsupplemented soils followed this same trend.

Root:shoot ratio

Root:shoot ratio, calculated on a dry weight basis, differed relatively little between the treatments and showed no obvious trend (Table 3.5.4). There was a relatively high root:shoot ratio of 1.10 :1 in soil exposed to microwaves for 180 sec and then supplemented with agricultural soil. This reflects the trends of increasing root dry weight and unchanging shoot dry weight with increasing microwave irradiation for this treatment.

Numbers of root tips

The mean number of root tips per seedling (Table 3.5.5.) was variable in the experiment as a whole but tended to increase with increasing exposure to microwaves. A statistically significant increase occurred after exposures of 60 sec for pooled supplementation treatments, but there was no significant difference between the mean number of root tips in soil treated for 60 and 180 seconds respectively. Supplementation of soil resulted in a significant decrease in the mean number of tips per seedling, averaged over microwave treatments. Inspection of Table 3.5.5. shows that these effects were consistent across the respective treatments. A comparison of numbers of root tips with root dry weight (Table 3.5.6.) showed no significant differences between exposure times or soil supplementation treatments. This is probably a reflection of the fact that the number of root tips per unit weight of root system was low even in the untreated soil, in contrast to the results of previous experiments.

Mycorrhizal development

Effects of treatments were assessed as described in the Farfield experiment (Section 3.3.1). Five main trends were observed as follows:

1)Overall, there was no significant effect of either exposure time or soil supplementation on the number of root tips that became mycorrhizal; this percentage ranged from 47 to 59% across the treatments (Table 3.5.7.)

2)No Lactarius-type mycorrhizas developed in any of the treatment combinations, despite the fact that soil in all tubes had been supplemented with *Lactarius* inoculum. However, five other mycorrhizal types developed on the roots of seedlings, presumably from naturally occurring inoculum in the soil. Three of these mycorrhizal types could be identified as belonging to *Thelephora terrestris*, *Hebeloma* sp. and *Cenococcum*; the other two types were distinctive and similar to the Ascomycete-type and H-type observed in the previous experiment with Bush soil (Section 3.4). Hebeloma-type, H-type and Thelephora-type mycorrhizas predominated on the root systems as a whole.

3)Except in the case of *Thelephora* , the proportions of mycorrhizal types were significantly affected by exposure to microwave irradiation, as explained in (5) below.

4)Supplementation with a small amount of fresh agricultural soil did not significantly affect the incidence of the mycorrhizal types, with the exception of *Cenococcum* which was significantly reduced by soil supplementation (Table 3.5.11).

5)Development of Hebeloma-type mycorrhizas was eliminated by

Tables 3.5.7 - 3.5.12
Mycorrhizal development on birch seedlings grown for 12 wk in Bush soil exposed to microwave irradiation for different times and then either supplemented or not supplemented with agricultural soil; means of 12 replicates (means of angular transformation in parenthesis).

Table 3.5.7
Percentage of root tips with mycorrhizas

Supplementation	Exposure (sec) 0	60	180	Row means	
None	51.0	51.2	46.9	(43.5)	
+ soil	59.1	46.5	50.8	(43.0)	SED (3.14)
					5%LSD -
Column means	(43.8)	(44.7)	(41.2)		
		SED	(3.84)		
		5%LSD	-		

Table 3.5.8
Percentage of mycorrhizal root tips attributable to Hebeloma

Supplementation	exposure (sec) 0	60	180	Row means	
None	32.4	40.9	0	(22.6)	
+ soil	49.6	65.8	0	(28.4)	SED (5.02)
					5%LSD -
Column means	(28.9)	(47.5)	(0.0)		
		SED	(6.15)		
		5%LSD	(12.30)		

Table 3.5.9
Percentage of mycorrhizal root tips atributable to Thelephora

Supplementation	Exposure (sec) 0	60	180	Row means	
None	7.1	7.0	14.1	(12.3)	
+ soil	10.8	6.0	25.9	(16.0)	SED (4.95)
					5%LSD -
Column means	(15.5)	(9.4)	(17.6)		
		SED	(6.07)		
		5%LSD	-		

Table 3.5.10
Percentage of mycorrhizal root tips attributable to H-type

Supplementation	Exposure (sec) 0	60	180	Row mean	
None	50.1	36.4	90.9	(63.1)	
+ soil	36.1	25.3	74.1	(50.2)	SED (6.15)
					5%LSD -
Column means	(44.5)	(30.8)	(71.4)		
		SED	(7.53)		
		5%LSD	(15.06)		
		1%LSD	(20.03)		

exposure of soil to microwaves for 180 sec in both supplemented and unsupplemented soil (Table 3.5.10) but was significantly stimulated (relative to that in unexposed soil) by microwave exposure for 60 sec. The same trend were apparent in the previous experiment with Bush soil (see Table 3.4.10), though in that experiment the stimulation by short exposure times was not significant. In contrast to Hebeloma-type, the occurrence of mycorrhizas of H-type did not differ significantly between untreated soil and soils treated for 60 sec, but there was a highly significant increase in the occurrence of H-type mycorrhizas when soil was treated for 180 sec (Table 3.5.10). In these respects, again, the results for supplemented and unsupplemented soil were similar.

The incidence of Thelephora-type mycorrhizas was not significantly affected by either soil supplementation or exposure to microwave irradiation (Table 3.5.9), in contrast to the behaviour of this fungus in the previous experiment involving Bush soil. However, examination of Table 3.5.9 shows that Thelephora-type mycorrhizas were more common (though not significantly so) in soil irradiated for 180 sec than in untreated soil or that exposed for 60 sec, and that this effect occurred in both the supplemented and unsupplemented soils.

Mycorrhizas of both *Cenococcum* and the Ascomycete-type were least common in soil exposed for 180 sec (Tables 3.5.11 and 3.5.12), but the differences in this respect were not significant because these mycorrhizal types occurred on only nine and four seedlings, respectively, in the experiment as a whole.

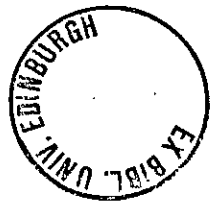


Table 3.5.11
Percentage mycorrhizal root tips attributable to Cenococcum

Supplementation	Exposure (sec) 0	60	180	Row mean	
None	1.6	14.5	0.3	(5.10)	
+ soil	0.8	0.4	0	(0.65)	SED (1.77)
					5%LSD(3.54)
Column means	(2.47)	(5.56)	(0.59)		
		SED	(2.17)		
		5%LSD	(4.33)		

Table 3.5.12
Percentage of mycorrhizal tips attributable to Ascomycete-type

Supplementation	Exposure (sec) 0	60	180	Row mean	
None	8.8	1.1	0	(2.1)	
+ soil	2.7	2.2	0	(3.2)	SED (2.24)
					5%LSD -
Column means	(5.7)	(2.2)	(0.0)		
		SED	(2.75)		
		5%LSD	(5.50)		

35.3. Summary.

Microwave irradiation of soil resulted in generally larger seedlings in terms of height, total root tips and root dry weight; but the most pronounced difference in these respects was between untreated soil and that treated for 60 sec, and there was no further significant effect when the microwave treatment was prolonged to 180 sec. Height, total root tips and shoot dry weight were significantly decreased by the addition of a fresh agricultural soil supplement. Neither the percentage of root tips that became mycorrhizal nor any of the main mycorrhizal types was affected significantly by the addition of a soil supplement. Of the main mycorrhizal types, the occurrence of *Hebeloma* was decreased by long exposure to microwaves, H-type was significantly increased and *Thelephora* was unaffected.

3.6. Experiment 4 – Comparison of three soils.

3.6.1. Introduction.

In this experiment soil was collected from three sites as follows.

1) A coal spoil heap at Newtongrange, Midlothian, subject to "after-burning" ; the soil was collected from the edge of the burning area beyond which the spoil was being colonised naturally by birch trees.

2)An ancient, relict birch woodland at Struan, Perthshire, the soil being obtained from the root zone of mature trees.

3)Birch woodland (more than 30 years old on a mixture of sands and gravels from a heathland at Hayes Common, south of London.

The soils were stored in polythene bags for one week then each was sieved < 2mm, mixed thoroughly and weighed into polypropylene tubes each containing 67 - 68g (Newtongrange) 48 - 49g (Struan) or 29 - 30g (London). The tubes were exposed to microwave irradiation for 0,20,40 and 80 sec, with 10 replicates per treatment. Aseptically germinated birch seedlings were planted, one per tube, and the tubes were incubated in a growth cabinet as for previous experiments. Results for parameters of seedling growth and mycorrhizal development are shown in Tables 3.6.1 – 3.6.12.

3.6.2. Results.

In this experiment, unlike all others described so far, analysis of variance sometimes revealed significant interaction effects in addition to the main effects attributable separately to soil types or microwave exposure times.

So it was sometimes necessary to perform one-way analyses of variance in order to separate treatment effects.

Seedling height

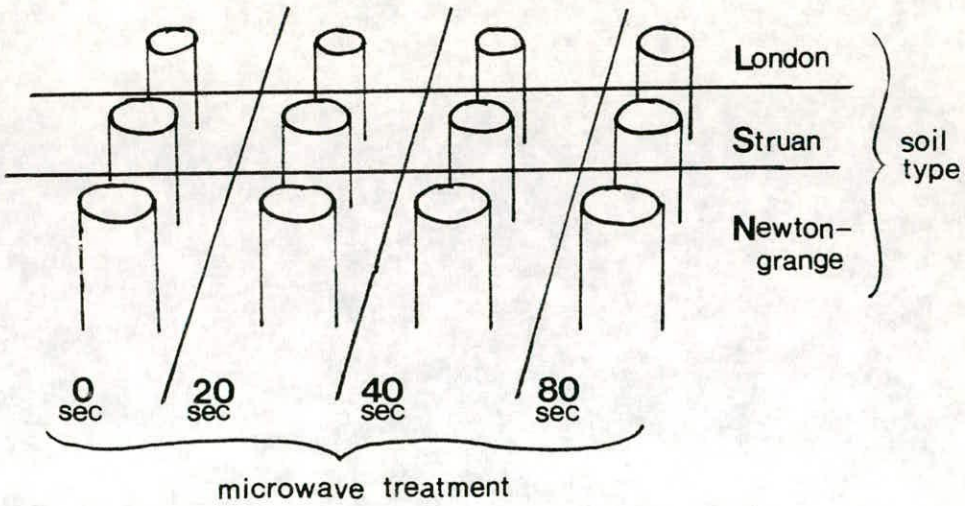
In the experiment as a whole, seedling height was significantly affected by microwave irradiation of soils and also differed significantly between soil types (Table 3.6.1). The pooled results for soil types showed that seedling height was significantly greater in soils treated for 20,40 and 80 sec than in untreated soil. Also, soil treated for 80 sec supported significantly taller seedlings than did that treated for 20 sec. However, inspection of Table 3.6.1 shows clearly that the soils behaved differently in these respects. In particular, there was evidence of a progressive growth response to increasing exposure times in Struan soil, a marked response to even short exposure times in London soil, but no beneficial growth response to microwave treatment of Newtongrange coal spoil. The combined data for microwave exposures showed that seedlings in Newtongrange soil were significantly smaller than those in London soil, and intermediate growth occurred in Struan soil. These trends are illustrated in Fig 3.6.1.

Shoot and root dry weights

The results for shoot dry weights broadly reflect those for seedling heights described above, in that there was a tendency for seedlings in Struan and London soil to increase in dry weight with increased exposure to microwaves but a tendency for dry weights of seedlings in Newtongrange soil to decrease with time of exposure to microwaves (Table 3.6.2). Again, seedling growth was

Fig 3·6·1

Representative sample of seedlings in each soil treatment in section 3·6



Tables 3.6.1 - 3.6.7
Growth parameters of birch seedlings grown for 12 wk in three soils exposed to microwave irradiation for different times; means of 10 replicates (means of log (x+1) transformation in parenthesis).

Table 3.6.1
Seedling height (mm)

Soil type	Exposure (sec) 0	20	40	80	Row mean	
N-grange	27.1	24.4	13.8	18.4	(1.27)	
Struan	12.2	25.4	41.0	54.4	(1.43)	SED (0.05)
London	31.3	76.4	71.7	96.3	(1.81)	5%LSD(0.09)
						1%LSD(0.12)
Column means	(1.31)	(1.52)	(1.54)	(1.64)		

SED (0.05)
5%LSD (0.11)
1%LSD (0.15)

Table 3.6.2
Shoot dry weight (g)

Soil type	Exposure (sec) 0	20	40	80	Row mean	
N-grange	13.1	8.0	0.7	5.5	(0.66)	
Struan	6.0	14.9	34.9	61.3	(0.99)	SED(
London	20.9	211.2	208.6	269.3	(2.01)	5%LSD(
						1%LSD(0.22)
Column means	(0.86)	(1.29)	(1.22)	(1.52)		

SED (0.12)
5%LSD (0.25)

1%LSD (0.33)

(N.grange = Newtongrange)

Table 3.6.3
Root dry weight (g)

Soil type	Exposure (sec) 0	20	40	180	Row mean	
N-grange	80.5	3.8	2.7	5.2	(0.69)	
Struan	17.8	5.7	10.2	27.8	(0.77)	SED (0.093)
London	9.3	119.0	107.3	137.0	(1.75)	5%LSD(0.186)
						1%LSD(0.247)
Column means	(0.75)	(1.09)	(1.11)	(1.33)		

SED (0.107)
5%LSD (0.214)
1%LSD (0.285)

Table 3.6.4
Ratio of root and shoot dry weight

Soil type	Exposure (sec) 0	20	40	80	Row mean	
N-grange	0.9	1.2	5.0	1.3	2·1	
Struan	1.9	1.3	0.6	0.5	1·07	SED 0·4
London	0.7	0.5	0.5	0.5	0·55	5%LSD 0·8
						1%LSD 0·9
Column means	1·18	1·0	2·03	0·78		

SED 0·74
5%LSD —

Table 3.6.5
Mean number of root tips per seedling

Soil type	Exposure (sec) 0	20	40	80	Row mean	
N-grange	55.1	21.2	22.5	32.0	(1.38)	
Struan	17.8	58.6	64.8	100.3	(1.62)	SED (0.06)
London	34.7	151.4	122.5	145.4	(1.94)	5%LSD(0.13)
						1%LSD(0.17)
Column mean	(1.41)	(1.68)	(1.69)	(1.81)		

SED	(0.07)
5%LSD	(0.15)
1%LSD	(0.20)

Table 3.6.6
Percentage of moribund root tips

Soil type	Exposure (sec) 0	20	40	80	Row mean	
N-grange	0	0	0	0	(0.0)	
Struan	0	0	0	0	(0.0)	
London	4.8	9.9	9.1	2.6	(9.1)	SED (1.84)
						5%LSD(3.68)
Column means	(4.0)	(14.7)	(11.6)	(6.1)		1%LSD(4.89)

SED	(2.12)
5%LSD	(4.25)
1%LSD	(5.65)

Table 3.6.7
Ratio of number of root tips : root dry weight

Soil type	Exposure (sec) 0	20	40	80	Row mean		
N-grange	12.5	12.7	12.5	15.8	13·3		
Struan	26.2	15.2	10.3	5.8	14·4	SED	2·24
London	5.4	1.4	1.3	1.1	2·5	5%LSD	4·52
						1%LSD	5·42
Column mean	14·7	9·8	8·1	7·6			

SED	2·45
5%LSD	4·95
1%LSD	5·92

significantly different between soil types, when the data for exposure times were combined. Root dry weights were affected similarly to shoot dry weights, except that there was no clear trend in Struan soil, and somewhat larger root systems occurred in untreated than in microwave-treated Newtongrange soil (Table 3.6.3). Analysis of variance revealed a significant interaction between soil type and microwave exposure with respect to root dry weights.

Root:shoot ratio

Root:shoot ratios, calculated in a dry weight basis, varied considerably between treatments (Table 3.6.4). Averaged for microwave exposures, there was no significant difference between root:shoot ratios for seedlings in Struan and London soils but the ratios in these soil types were significantly lower than for Newtongrange soil. Averaged for soil type, there was no significant effect of exposure to microwaves on the root:shoot ratio. However Table 3.6.4 shows a tendency for the ratio to decrease with increasing exposure time in the Struan and London soils, but to vary considerably and with no obvious pattern in Newtongrange soil.

Numbers of root tips

The mean number of root tips per seedling was variable in the experiment as a whole but tended to increase with increasing exposure of soils to microwaves, a statistically significant increase occurring after exposures of 20 seconds or more for data averaged for soil type (Table 3.6.5). As shown in the table, however, this trend was not evident in Newtongrange soil. For data averaged for microwave treatments, there were highly significant differences

between all three soil types. Also of interest is the observation that a proportion of the root tips were moribund on seedlings grown in London soil but not in the other soils (Table 3.6.6); there were some significant differences between microwave exposure times in this respect. The number of root tips expressed as a proportion of root dry weight was significantly decreased by exposure of soils for 80 sec compared with shorter exposure times, or none, when the results were combined for soil types. But again the seedlings in Newtongrange soil did not follow this trend and the ratio in this soil even increased slightly with 80 sec exposure. For results averaged for microwave exposures, the ratio for seedlings in Newtongrange and Struan soils were not significantly different but both were significantly greater than for seedlings in London soil (Table 3.6.7).

Mycorrhizal development.

Effects of treatments were assessed as described in the Farfield experiment (Section 3.3.1). A number of trends were observed, as follows:

1)Overall, there was no significant effect of soil type on the proportion of root tips that became mycorrhizal, averaged over microwave exposure times (Table 3.6.8). However, exposure to microwave irradiation, analysed across the soil types, did significantly affect mycorrhizal development, which was less for any exposure time than in untreated soil. This trend was apparent in the results for each soil type.

2) A range of mycorrhizal types developed on the roots of seedlings grown in all soil types. This range varied from soil to soil and in three out of eight instances, involving mycorrhizas of *Thelephora*, *Paxillus* and *Cenococcum*, was significantly affected by exposure to microwave irradiation as explained below.

Tables 3.6.8 - 3.6.12
Mycorrhizal development on birch seedlings grown for 12 wk in three soils exposed to microwave irradiation for different times; means of 10 replicates (means of angular transformation in parenthesis).

Table 3.6.8
Percentage of root tips with mycorrhizas

Soil type	Exposure (sec) 0	20	40	80	Row mean	
N-grange	67.1	25.3	13.3	46.4	(26.6)	
Struan	48.7	9.0	23.5	39.4	(28.9)	SED (5.1)
London	43.1	38.4	29.7	14.5	(25.7)	5%LSD -
Column means	(42.5)	(27.3)	(19.7)	(25.7)		

SED	(5.8)
5%LSD	(11.7)
1%LSD	(15.6)

Table 3.6.9
Percentage of mycorrhizal root tips attributable to Thelephora

Soil type	Exposure (sec) 0	20	40	80	Row mean	
N-grange	0	25.6	100.0	100.0	(53.7)	
Struan	88.5	54.0	100.0	100.0	(81.5)	SED (5.5)
London	9.9	8.6	16.1	40.7	(13.3)	5%LSD(10.9)
						1%LSD(14.6)
Column means	(30.4)	(38.0)	(63.9)	(65.6)		

SED (6.3)
5%LSD (12.7)
1%LSD (16.9)

Table 3.6.10
Percentage of mycorrhizal root tips attributable to Lactarius

Soil type	Exposure (sec) 0	20	40	80	Row mean	
N-grange	3.7	30.2	0	0	(10.2)	
Struan	0	5.4	0	0	(0.8)	SED (4.8)
London	21.9	16.1	5.8	11.8	(10.5)	5%LSD(9.6)
Column means	(12.8)	(8.0)	(2.4)	(4.4)		

SED (5.5)
5%LSD -

3) Three mycorrhizal types developed on the roots of seedlings in Newtongrange soil, presumably from naturally occurring inoculum; the three types could be identified as belonging to *Thelephora terrestris*, *Lactarius* spp. and *Paxillus involutus*. *Thelephora* predominated on root systems in this soil, accounting for all mycorrhizal development on seedlings in soil exposed to microwaves for 40 and 80 sec (Table 3.6.9). In contrast, the root systems of seedlings in untreated soil did not bear mycorrhizas of *Thelephora* and instead bore mycorrhizas almost exclusively of *Paxillus*, (Table 3.6.11) though a small proportion of root tips were infected with *Lactarius* (Table 3.6.10). Roots of seedlings in soil treated with 20 sec exposure bore mycorrhizas of *Thelephora*, *Lactarius* and *Paxillus* - the only treatment in which all three of these mycorrhizal types occurred.

4)Three mycorrhizal types were observed on the roots of seedlings grown in Struan soil, presumably from naturally occurring inoculum; the three types were identified as belonging to *Thelephora terrestris*, *Lactarius* spp. and *Cenococcum* . As with Newtongrange soil, Thelephora-type mycorrhizas occurred to the exclusion of all other types on the root systems of seedlings in soil exposed to microwaves for 40 and 80 sec. However, unlike the case with Newtongrange soil, seedlings in untreated Struan soil were infected predominantly by *Thelephora* and bore some mycorrhizas of *Cenococcum*.

Seedlings in soil treated for 20 sec bore mycorrhizas of *Thelephora* with a small proportion of root tips attributable to A-type (unidentified) (Table 3.6.12).

5) Seven mycorrhizal types were observed on the roots of seedlings grown in London soil, presumably from naturally occurring inoculum. Three of the

Table 3.6.11
Percentage of mycorrhizal root tips attributable to Paxillus

Soil type	Exposure (sec) 0	20	40	80	Row means	
N-grange	95.3	44.2	0	0	(26.2)	
Struan	0	0	0	0	(0.0)	SED (3.4)
London	0	0	0	0	(0.0)	5%LSD(7.6)
Column means	(68.2)	(36.6)	(0.0)	(0.0)		

SED (3.9)
5%LSD (13.5)
1%LSD (18.0)

Table 3.6.12
Percentage of mycorrhizal root tips attributable to A-type

Soil type	Exposure (sec) 0	20	40	80	Row means	
N-grange	0	0	0	0	(0.0)	
Struan	0	0	0	0	(0.0)	SED (5.98)
London	(47.6)	(38.7)	(55.7)	(52.6)	(48.6)	5%LSD -

SED (6.9)
5%LSD -

types could be identified as belonging to *Thelephora terrestris*, *Lactarius* and *Cenococcum* spp., but the other four types were distinctive.Three unidentified types occurred too infrequently for statistical analysis (on a total of five plants) but the fourth, called "A-type", predominated on the root systems. A-type, Thelephora-type and Lactarius-type mycorrhizas occurred in untreated soil and in all microwave exposure treatments. Thelephora-type mycorrhizas tended to increase, and there was a tendency for Lactarius-type mycorrhizas to decrease, with increased exposure time.

3.6.3. Summary.

Soil type had a significant effect on each parameter measured. Seedlings in London soil tended to be the largest, followed by those in Struan soil and then those in Newtongrange soil. The growth of seedlings in Newtongrange soil tended to be unaffected by increasing exposure of the soil to microwave irradiation but seedlings in both Struan and London soils tended to grow larger as irradiation of the soil increased. Whereas the degree to which the root systems became mycorrhizal was not significantly different between soil types, the development of particular mycorrhizal types did differ. Seedlings in Newtongrange and Struan soil became infected predominantly by *Thelephora*, particularly at the higher exposure times, whereas the development of mycorrhizas in London soil was more evenly distributed between three types, namely A-type, *Thelephora* and *Lactarius*, at all exposure times.

3.7. Microbial populations in microwave-treated and untreated soils.

3.7.1. Introduction.

In each of the four microwave experiments described so far, one additional tube of soil was prepared for each exposure treatment and was used to assess the effects of treatments on the microbial populations.

As soon as possible after microwave exposure, one gram of soil was removed from each tube, observing aseptic technique, and placed into 9 ml sterile distilled water in a McCartney bottle. The bottles were shaken on a wrist action shaker for 5 min and the original suspensions were used to prepare dilution series by serially transferring 1 ml into 9 ml batches of sterile distilled water. An aliquot (0.1 ml) of each dilution was spread on to each of three replicate plates of one-quarter strength Tryptic-Soy Agar for detection of bacterial populations, and similarly on to Czapek-Dox Agar with streptomycin (100 $\mu g ml^{-1}$) for the detection of fungal populations. The plates were incubated at 20° C for up to 14 days, the colonies being counted where appropriate and identified where possible.

3.7.2. Bacterial populations.

The main types of bacteria occurring in the five soils were categorised as shown in Table 3.7.1, and the occurrence of these types in soils exposed to microwave irradiation is shown in Table 3.7.2. The total bacterial population, estimated by dilution plating of the soils, is shown in Table 3.7.3, and the estimated populations of spore-formers (types c,e,h, i and j) and a distinctive non-spore-forming type (type g) that resembled *Cytophaga* are shown in Table 3.7.4. Results for Farfield soil have been excluded from the tables, because this

Table 3.7.1
Characteristics of bacterial types growing on Tryptic-soy agar from soils exposed to microwave irradiation for various times.

Type	Colour	Colony appearance	Cell shape	Phase-bright spores	Gram reaction
a	cream	transparent	large rods	0	+
b	white	smooth	cocci	0	+
c	yellow-cream	matt	large rods	terminal	±
d	cream	shiny	cocci	0	+
e	cream	flat, spreading	large rods	central	+
f	yellow-cream	shiny	short rods	0	-
g	yellow	transparent	long rods(motile)	0	+
h	grey-green	slimy	large rods	terminal	+
i	white	dry, crusty	large rods	terminal	+
j	colour-less	transparent	short rods	terminal	+
k	*Bacillus cereus* var. *mycoides*				
l	beige-grey Actinomycete				

* Gram reaction +, positive; -, negative; ±, variable

Table 3.7.2
Predominant bacterial types (as defined in Table 3.7.1) in order of abundance, observed by dilution-plating on Tryptic-Soy agar, of soils exposed to microwave irradiation for different times.

Microwave exposure (sec)	Soil type (and relevent section of thesis) Bush (3.4)	Bush (3.5)	N.grange (3.6)	Struan (3.6)	London (3.6)
0	c,i,f,k l,g	c,f,i,k l,g	a,d,k,g j	d,f,k,a c	d,h,g,j b,f,a,i
20	c,i,g,f k,l	-	a,b,d,c g	d,e,c,k j	a,k,b,f j,h,d
40	c,f,i,k l	-	a,k	e,d,e,k	d,a,k,f h,j,b
60	c,i,k,l	c,i,k,l	-	-	-
80	-	-	a	a,c,k,d	d,f,k,c
180	i,k	i,k	-	-	-

(-, no experimental treatment)

Table 3.7.3
Numbers of bacteria (x 10^3 g^{-1}) estimated by dilution-plating on Tryptic-Soy agar, in soils exposed to microwave irradiation for different times.

	Soil type (and relevant section of thesis)					
Microwave exposure (sec)	Farfield (Sect 3.3)	Bush (Sect 3.4)	Bush (Sect 3.5)	N.grange (Sect 3.6)	Struan (Sect 3.6)	London (Sect 3.6)
0	2240	6390	1270	440	1100	1160
15	1600	-	-	-	-	-
20	-	5300	-	91	1400	250
30	2040	-	-	-	-	-
40	-	-	-	1	6	330
50	12	-	-	-	-	-
60	-	18	104	-	-	-
80	4	-	-	1	2	2
180	-	3	1	-	-	-

(-, no experimental treatment)

Table 3.7.4
Numbers of spore-forming (types c,e,h,i,j) and Cytophaga-type (type g) bacteria (x 10^3 g^{-1}) estimated by dilution plating on Tryptic-Soy agar, in soils exposed to microwave irradiation for different times.

Microwave exposure (sec)		Soil type Bush	N.grange	Struan	London
0	Spore-formers	1760	8	500	170
	Cytophaga-type	100	20	0	40
20	Spore-formers	790	0.7	50	12
	Cytophaga-type	60	0.1	0	0
40	Spore-formers	-	2	0.1	10
	Cytophaga-type		0	0	0
60	Spore-formers	1.2	-	-	-
	Cytophaga-type	0			
80	Spore-formers	-	0	0.1	0.1
	Cytophaga-type		0	0	0
180	Spore-formers	0.2	-	-	-
	Cytophaga-type	0			

(-, no experimental treatment)

soil was used for the first experiment of this type and detailed assessments of its bacterial population were not made.

Total bacterial counts in the absence of microwave treatment were similar over the range of soils ($1x10^6$ to $6x10^6$ colony-forming units g^{-1}) with the exception of Newtongrange soil in which numbers were approximately ten times lower. In each soil the bacterial count was reduced by microwave treatment but bacteria were not eliminated by even 80 sec or 180 sec exposure to microwaves. Nevertheless, for each soil, microwaving for 80 or 180 sec resulted in an approximately 1000-fold reduction in the detectable bacterial population.

Of the bacterial types, some were evidently less sensitive to microwave treatment than were others. In particular, the types that predominated at high exposures tended to be spore-formers (types c,e,h,i,j and k) and this, together with their gram reaction, cell morphology and colony appearance, suggested that they were *Bacillus* spp, which are notable for their heat resistance. Although spores were not observed in cultures of type a, characteristics such as cell size and shape and colony appearance indicated that this type also is referable to *Bacillus*. However, some bacterial types that occurred in soils subjected to long microwave exposure times were not of Bacillus-type; examples of this include types d and f in Struan and London soils exposed for 80 sec, but type d was less common in this microwave treatment (estimated population, 10 cfu g^{-1}) than in the 40 sec treatment (500 cfu g^{-1}). Bacterial type g, with features resembling those of *Cytophaga*, was among the most heat-sensitive, being eliminated after 40 sec exposure of soil to microwave irradiation.

3.7.3. Fungal populations.

Fifteen recognisable types of fungus were seen in soil dilutions plated on to Czapek-Dox agar with streptomycin, as shown in Table 3.7.5. Of these types, *Penicillium*, *Cladosporium*, *Gliocladium* and *Trichoderma* occurred most frequently, which is predictable in view of their common occurrence in soil (Domsch,Gams & Anderson,1980) and profuse sporulation which favours their appearance on dilution plates (Warcup, 1983). However, only *Penicillium* and *Cladosporium* occurred in all of the soils tested. There was some evidence of differences between soils. For example, the diversity of fungal types was less in soils from Newtongrange, Struan and the London sites than in soils from Bush and Farfield. Newtongrange coal spoil, in particular, contained few detectable fungi, probably reflecting its low organic matter content and the adverse characteristics of the coal spoil site.

The fungal population was markedly affected by exposure of soils to microwaves, as reflected in a progressive decrease in the number of fungal types with increasing length of exposure for all soils. Indeed, no fungus was detected in Newtongrange and Struan soils after 40 sec exposure, and none in London soil after 80 sec exposure. Similarly, few fungal colonies, consisting exclusively of *Penicillium* and *Cladosporium*, were seen in dilutions of Bush and Farfield soils after 80 sec exposure. Single colonies of these fungi still occurred after 180 sec exposure, as shown in Table 3.7.5, but it is possible that these colonies arose from contaminating spores.

Unlike the case with bacteria, none of the fungi listed in Table 3.7.5 is noted for heat-resistance of its spores. Instead, it is concluded that the persistence of some fungi after the longer exposure times was a reflection

Table 3.7.5
Types of fungal colonies, as defined overleaf appearing on dilution plates of soils exposed to microwave irradiation for different times.

Microwave exposure (sec)	Soil type Farfield	Bush (1)	Bush (2)	N.grange	Struan	London
0	a,b,d,f g,h,i,j k	a,d,e,f g,h,j,k l,m,n	a,b,c,f j,k,l,m n	a,c,i,k	a,b,c,i k,l	a,c,i,j k
15	a,b,d,e g,h,i,o	-	-	-	-	-
20	-	a,b,d,e	-	a,c,k	a,i,j,l n	a,j,k
30	a,b,d,h i,l,m,n	-	-	-	-	-
40	-	-	-	none	none	a,j,k,n
50	a,g,h,l	-	-	-	-	-
60	-	a,b,c,d h,i	a,b,f,k l,n	-	-	-
80	a	a,k	-	none	none	none
180	-	a,k (*)	k (*)	-	-	-

-. no experimental treatment; (*), infrequent occurrence which may have arisen from laboratory-borne contamination.

Fungal types and notations for Table 3.7.5

a *Penicillium*
b *Cladosporium*, *Alternaria* or *Stemphilium*

c *Fusarium*
d *Epicoccum*
e *Cylindrocarpon*?
f *Thamnidium*
g *Cephalosporium*
h *Alternaria* or *Helminthosporium*
i *Trichoderma*
j *Gliocladium*
k *Cladosporium*
l *Mucor*
m *Botryoderma*?
n *Phoma*
o *Myrothecium*

largely of the size of the initial population of the spores and thus the increased chance that some of these spores would persist in local pockets of soil during the longer exposure times. However, a chance observation suggested that the methodology used in this work was inappropriate in some respects. A few of the dilution plates with low colony counts had been inadvertently left on the laboratory bench after being assessed, and some weeks later a perithecium-bearing colony of *Chaetomium* was observed on one plate. As ascospores of several fungi (notably those of *Neurospora* spp. and of *Rhizina undulata*) are known to be activated by heat but otherwise show constitutive dormancy, it is possible that ascospores would have survived microwave treatment of soils but the ascosporic fungi were not detected because of competition from other fungi or the relatively short incubation times before the colony counts were made.

3.8. Discussion.

In this series of experiments, five different soils were exposed to microwave irradiation for up to 180 sec prior, in some cases, to supplementation with mycorrhizal inoculum or a small amount of untreated agricultural soil. Birch seedlings were then planted and sampled after a standard period of 12 weeks, when their growth and mycorrhizal status were assessed. Despite variations between the results of individual experiments, a number of common features emerged that are worthy of discussion.

Seedling growth responses to microwave treatments

In four of the five soils, seedlings showed a significant growth response to prior microwave treatment of the soil. The exception was

Newtongrange coal spoil which will be considered later. The growth response was not unexpected; it had previously been described for soybean, sorghum and Sudan-grass when grown in microwave -treated soil (Ferriss,1984) and it parallels numerous other reports in which plant growth has been shown to be increased by treatments that partially sterilize soils. Such treatments include aerated steam (Baker,1970), solarization (Katan,1980), and fumigation with chemicals (Altman & Tsue,1965; Benzian,1965; Turner & Goring,1966; Salt,1969; Ladd *et al*,1977; Jenkinson,1976; Cook & Haglund,1982).

Sometimes such growth responses are attributed to the release of nutrients from microorganisms killed by the soil treatment or released from complexes with soil organic matter (Waksman,1932; Alexander,1961; Ladd *et al*,1976; Jenkinson,1976; Jenkinson, Powlson & Wedderburn, 1976). In other instances the growth responses are thought to result from destruction of root-infecting pathogens (Salt,1969; Katan,1980; Pullman, DeVay & Garber,1981; Cook & Haglund,1982; Ferriss,1984). Recent work suggests yet another way in which partial sterilisation might enhance plant growth. A complex of yield-depressing minor pathogens (Salt,1970) and deleterious rhizosphere bacteria (Suslow & Schroth, 1982; Geels & Schippers,1983) has been detected in agricultural soils; some of these bacteria have been found to be heat-sensitive pseudomonads (Geels & Schippers,1983). Much of this work has involved agricultural crops rather than trees, but it has long been known that tree seedlings also benefit from treatments such as soil fumigation (Benzian,1965). The report by Benzian (1970) is particularly relevant because it involved studies of some of the possible reasons for seedling growth increases, such as a reduction in the populations of pathogens (Ram Reddy,Salt & Last,1964;Griffin,1965) and nematodes (Goodey,1965) and the provision of an

energy source for microorganisms (Benzian,1965). But these were rejected as primary causes and the reason for the growth increases remained unknown. My work was not designed to investigate the reasons for plant growth responses to microwave irradiation, but this technique does offer another potential tool for investigating the underlying mechanisms.

Comparison of the results of the microwave experiments on the different soils reveals an interesting point, namely that a seedling growth response often occurred after a given length of microwave treatment and thereafter there was little or no further growth response to longer exposure times. This was seen, for example, in Farfield, Bush and London soils (Sections 3.3.2, 3.4.2 and 3.6.2 respectively) although in Struan soil (Section 3.6.2) there was evidence of a progressive seedling response to length of microwave treatment and in Newtongrange soil (Section 3.6.2) there was no response at all. The times of microwaving after which the major growth response occurred varied considerably between soils, being as little as 20 sec in London soil compared with 50 - 60 sec for Bush and Farfield soils. However, inspection of Table 3.2.1 (showing the temperatures attained in each soil type after each appropriate exposure time) provides a possible explanation of this, because the London soil reached a high temperature (70 °C) after much shorter periods of microwaving than did the Bush and Farfield soils. Indeed, it is seen that the length of microwave treatment neccessary to cause the growth response was, in each of the three soils, the time needed to raise the soil temperature to 70 - 80° C (60 sec giving 82 or 83° C for Bush soil, 50 sec giving 79 °C for Farfield soil and 20 sec giving 70° C for the London soil). Vela & Wu (1979) and Ferriss (1984) have discussed the means by which microwave irradiation causes changes in microbial populations of soils and have concluded that the main effect is likely to be through heating. In the conditions of my experiments, soils raised to a

temperature of , for example, 80° C by 50 - 60 sec exposure to microwaves were found to cool fairly quickly (e.g. to room temperature within 10 min) after removal from the oven and so any heating effect resulting in an improvement in seedling growth would have lasted only for a short time. That such relatively short exposures could significantly influence microbial populations is evidenced by the results in Table 3.7.3. The shortest microwave exposure times after which significant growth responses were observed were also the shortest exposure times (of those tested) that caused substantial reductions in bacterial populations. They also caused some simplificaton of the fungal populations in terms of species diversity. Yet the growth response in London soil exposed to microwaves for 20 sec coincided with only a 5-fold reduction in the total bacterial count (Table 3.7.3), which could hardly be considered large enough to account for the growth response. Possibly the significant point in this respect is that 20 sec exposure of London soil, like 60 sec exposure of Bush soil, led to the apparent elimination of some non-spore forming bacteria of which Cytophaga (type g) was an example (Table 3.7.2). It had been hoped that the reintroduction of a small amount of untreated soil to microwave-treated soil (Section 3.5) might help to identify the roles of heat-sensitive bacteria in plant responses to microwave irradiation. Such "re-seeding" of the soil did, indeed, reduce plant growth compared with that in non-supplemented soil, but there was evidence that this occurred even in the non-microwaved soil (Table 3.5.1) so the results are difficult to interpret.

Three other features of the seedling growth responses to microwave treatment merit comment. Firstly, there was a progressive response to exposure in Struan soil, which cannot be explained but suggests the need for further study of this soil, which behaved differently from the others. Secondly, there was an anomalous result for Bush soil, in which exposure for 180 sec caused poorer

seedling growth than did exposure for 60 sec. This effect was seen in both experiments involving Bush soil (Tables 3.4.1 and 3.5.1) but, remarkably, was found only if the soil was not then supplemented in some way: the addition of either a small amount of unsterilised soil or of *Amanita* inoculum (not, itself, effective in forming mycorrhizas) counteracted this effect. Further study would be required to investigate the reasons for this. Thirdly, as was noted earlier, there was no plant growth response to microwave treatment of Newtongrange soil. The most likely reason for this is that the coal spoil tends to be an adverse medium for seedling growth with very low levels of organic matter, low availability of the major mineral nutrients and possibly high levels of potentially toxic elements such as zinc and other heavy metals. Its low microbial population (Table 3.7.3) was a reflection of the low degree of biological activity in this substrate. Indeed, in so far as seedling growth response to microwave irradiation might be and indication of the potential of different soils to support plant growth, the data in Tables 3.3.2, 3.4.2, 3.5.2 and 3.6.2 suggest that Bush, Farfield and London soils have high potentials (maximum shoot dry weights were in the range 180 - 270 mg) whereas Struan soil (maximum shoot dry weights approximately 60 mg) and Newtongrange soil (maximum shoot dry weight 13 mg) have low potentials though probably for different reasons.

Changes in root dry weights with microwave treatment in general paralleled the changes in shoot dry weight, such that root:shoot ratios were changed little by exposure of soil to microwaves. Nevertheless, in most experiments the numbers of root tips ("short roots") potentially available for colonisation by mycorrhizal fungi increased less than did the root dry weight when plants were exposed to microwaves (Tables 3.3.3, 3.4.3, 3.5.3 and 3.6.3). Unfortunately, no assessment was made of leaf area and thus potential photosynthetic activity of the plants. This should be done in future experiments

of this type in order to see if the potential rate of shoot growth might be limited by the availability of short roots, in which case an assessment could be made of the potential dependence of the plants on mycorrhizal activity.

Responses of mycorrhizal fungi to microwave irradiation

In three of the four experiments reported here, microwave treatment of soil did not significantly reduce the number and diversity of mycorrhizal types on plants. (Tables 3.3.7, 3.4.7 and 3.5.7), the exception being the experiment involving Newtongrange, Struan and London soils (Table 3.6.8) in which, combined, there was a significant reduction in mycorrhizal status caused by microwave irradiation. The lack of response in three of the experiments is, at face value, surprising because it seems to indicate persistence of mycorrhizal inoculum at temperatures at which propagules of other fungi were killed (Table 3.7.4). However, an alternative explanation, which is preferred, is that the soils became recolonised to at least some degree by spores of mycorrhizal fungi such as *Thelephora terrestris*. This species, in particular, is a common glasshouse "contaminant" at Bush Estate where the experiments were performed and its spores might have contaminated the tubes of soil during preparation or when the growth cabinets were opened for inspection or watering. Another possibility, which similarly cannot be excluded, is that cross-infection occurred between the tubes of soil, which were randomised in propagator trays; such cross-infection possibly occurred by movement of small arthropods between non-microwaved and microwaved tubes,although there is no direct evidence for this suggestion.

Analysis of the effects of microwave irradiation on individual mycorrhizal fungi is complicated by the fact that different exposure times resulted in a) differences in the maximum temperature reached by soil and b)

inevitably, differences in the length of time during which the soils were subjected to high temperatures. So there was most probably a "time x exposure" interaction affecting the soil inoculum, as has been recorded by others for aerated steam treatment of soil (Baker & Cook,1983), for soil solarisation (Pullman, DeVay & Garber ,1981) and for effects of fumigants such as chloropicrin and methyl bromide (Munnecke, Bricker & Kolbezen,1978). Analyses are complicated still further by the fact that different soils were heated to different extents by any single exposure time (Table 3.2.1) and probably also cooled at different rates after irradiation. None of these interpretational problems could easily have been overcome by altering the experimental design. Furthermore it was necessary to use a bioassay to detect surviving inoculum and its ability to colonise root systems, and this in turn might have been influenced by alterations in the populations of other microorganisms, including other mycorrhizal fungi, and by changes in nutrient availability to the plants. For all these reasons, caution is needed in interpreting the results. In the discussion below, attention will be focused first on soils that were not supplemented with mycorrhizal inoculum and then the effects of supplementation will be considered.

That inoculum of at least some mycorrhizal fungi was susceptible to microwave irradiation is indicated by the substantial reductions in incidence of mycorrhizas of these fungi as a result of microwaving. For example, *Hebeloma* was evidently eliminated or at least markedly reduced by 180 sec treatment of Bush soil in two experiments (Tables 3.4.10 and 3.5.8) although it was largely unaffected by 60 sec exposure in both experiments. There were no corresponding data for Farfield, Newtongrange, Struan and London soils because Hebeloma-type mycorrhizas did not develop in these, even if the soils were not irradiated. The evidence thus suggests that *Hebeloma* was a resident mycorrhizal type in Bush soil, rather than an air-borne contaminant (in which case it might have occurred

in a wider range of soils) and that its inoculum was resistant to 60 sec irradiation (82° C). Mycorrhizas of "H-type" also occurred only in Bush soil, suggesting that the fungus responsible for these is resident in this soil. However, the incidence of H-type mycorrhizas differed substantially from that of Hebeloma mycorrhizas. Irradiation for even 180 sec did not eliminate H-type mycorrhizas. On the contrary, irradiation for 180 sec significantly increased the incidence of H-type mycorrhizas in one of the two experiments involving Bush soil (Tables 3.4.9 and 3.5.11).

In Farfield soil, the incidence of the predominant and presumably indigenous Y-type mycorrhizas was markedly reduced by 80 sec (95 °C) but not 50 sec (79° C) exposure, although these mycorrhizas still accounted for roughly 25% of all mycorrhizal root tips after 80 sec exposure (Table 3.3.9). Comparison with the data for *Hebeloma* above (see Tables 3.4.10 and 3.5.8) suggests that *Hebeloma* and the Y-type fungus are more or less equally susceptible to microwaving, and more so than the H-type fungus. *Paxillus* (in Newtongrange soil) and *Cenococcum* (in Struan soil) seemed also to be sensitive to irradiation, being eliminated by 40 sec exposure, which involved heating to maxima of 91 and 93° C respectively in these soils.

In Newtongrange soil *Lactarius* was also eliminated by 40 sec exposure (Table 3.6.10), but remarkably it was not eliminated by even 80 sec exposure (>95° C) in London soil (Table 3.6.10). It seems unlikely that the soil properties *per se* would have such a marked effect in protecting inoculum from heat inactivation, but the London soil, which prior to sieving was seen to contain large quantities of *Lactarius*-infected roots, may have contained sufficient inoculum to enable some to survive in pockets of organic matter in the soil. An alternative possibility, consistent with data in Table 3.6.10, is that the more "aggressive"

mycorrhizal fungus, *Thelephora*, was able readily to recolonise Newtongrange soil after microwaving and prevented any surviving inoculum of *Lactarius* from establishing mycorrhizas in this soil. The incidence of Thelephora-type mycorrhizas was enhanced by long irradiation of London soil, but not to such a degree as in Newtongrange soil (Table 3.6.9). The incidence of type-A mycorrhizas, which occurred only in London soil, was reduced but not eliminated by 80 sec exposure to microwaves. Type-A thus behaved like type-Y in Farfield soil.

Except for *Thelephora*, very few mycorrhizal types were more common in long-irradiated soils. These types were H-type in Bush soil (one experiment) and an ascomycete-type in Farfield soil (Tables 3.4.9 and 3.3.10 respectively). Mycorrhizas of a different ascomycete were, however, reduced by microwave exposure in Bush soil (Table 3.5.12). Yet, short exposure times were evidently favourable to some mycorrhizal types; the incidence of *Hebeloma* was significantly increased over that in untreated soil by relatively short exposure times in Bush soil (Tables 3.4.10 and 3.5.8), as was that of *Cenococcum* also in Bush soil (Table 3.5.11). Perhaps these fungi were favoured by some reduction in the activities of other microorganisms or other mycorrhizal fungi.

Summarising these findings, with due regard to the interpretational difficulties mentioned earlier, it seems that *Hebeloma*, the Y-type mycorrhizal fungus, *Paxillus*, *Cenococcum* and the ascomycete in Bush soil were among the most heat-sensitive mycorrhizal fungi, whereas H-type and A-type (in Bush and London soils respectively) were among the somewhat less sensitive, as was *Lactarius* in one (London) soil. The incidence of Thelephora mycorrhizas was enhanced in most soils subjected to long microwave exposure times, though whether it was resident or an air-borne contaminant is open to question. An

ascomycete in Farfield soil was favoured by long microwave exposure times and some other mycorrhizal fungi were favoured by short exposure times in comparison with their incidence in non-treated soil.

Effects of soil supplements

Supplementation of Bush soil with inoculum *Amanita muscaria* or supplementation of Farfield soil with inoculum of *Lactarius* did not increase the total mycorrhizal status of the plants but did, in some instances, alter the pattern of occurrence of different mycorrhizal types.

Lactarius successfully established mycorrhizas from added inoculum in Farfield soil, but not in Bush soil to which it was added in one (Section 3.4) of two experiments. In the Farfield soil, *Lactarius* became one of the dominant mycorrhizal types in both irradiated and non-irradiated treatments to which its inoculum was added (Table 3.3.8), but its incidence was significantly increased if the soil had been irradiated for 30 sec or more. It is notable that 30 sec exposure was the minimum period (of those tested) required for a significant seedling growth response to microwave irradiation in this soil. This implies a direct or indirect relationship between the factors that enhanced infection by *Lactarius* and those that benefited seedling growth. Another notable feature of the results in Farfield soil was that inoculation with *Lactarius* significantly reduced the incidence of mycorrhizas of Y-type and an ascomycete, but not of *Thelephora*. Inoculum of *Amanita* was ineffective in forming mycorrhizas in Bush soil at any exposure time (Section 4.4), and had no effect on the incidence of other mycorrhizal types. This fact, coupled with the finding that inoculum of *Lactarius* was ineffective in Bush soil (Section 3.4), leads to two provisional conclusions. Firstly, in the conditions of these experiments, Bush soil was evidently less

conducive to the development of Lactarius-type mycorrhizas than was Farfield soil. *Lactarius pubescens* has been categorised as a late-stage mycorrhizal fungus in extensive work with Bush soil (Deacon *etal*,1983;Fleming,1983a,1984,1985; Last & Fleming,1985;Fox,1983,1986) but *Lactarius* species have been found to develop mycorrhizas more readily in some other soils, notably in Struan Wood (Fleming *et al*,1986) and in a commercial forest site at Elibank (F.M.Fox, pers.comm.), where it may act more as an early-stage mycorrhizal fungus on seedlings. Further comparisons of Bush and Farfield soils seem appropriate in this respect. Secondly, from my results it is clear that microwave irradiation *per se* does not neccessarily enable proposed late-stage mycorrhizal fungi such as *Lactarius pubescens* and *Amanita muscaria* to develop mycorrhizas from inoculum supplements in soils in which they would otherwise not develop. But microwave irradiation can enhance the degree of development of such fungi (or at least of *Lactarius pubescens*) in soils in which they would otherwise establish at very low levels.

Supplementation of microwave-treated soil with a small amount of "fresh" soil (Section 3.4) did not significantly influence mycorrhizal development, except in one instance in which it reduced the degree of development of Cenococcum-type mycorrhizas (Table 3.5.11).But these represented only a small proportion of the total mycorrhizal population in this experiment, so the effect of supplementation overall was minimal. This was in some respects a disappointing result, because the reintroduction of specific microorganisms or of untreated soil after partial soil sterilisation has proved to be a useful experimental approach to the study of microbial activities in soil - for example in relation to disease-suppressiveness (Cook & Baker, 1983). Evidently, more work is required in this field before the technique can be used as an experimental tool in mycorrhizal research.

A final point worthy of comment concerns seedling growth responses to soil supplementation. There was no significant seedling response to supplementaion with *Lactarius*, even when this fungus developed mycorrhizas (Section 3.4). There was a significant increase in seedling growth when soil was supplemented with *Amanita* (Section 3.4), even though this fungus did not develop mycorrhizas. However, inspection of Tables 3.4.1 - 3.4.3 shows that this effect was due almost entirely to differences in soil exposed to 180 sec irradiation when the seedlings in non-supplemented soil showed anomalously poor growth. Lastly, supplementation with a small amount of unsterilised soil (Section 3.5) caused a significant reduction in seedling growth. The reasons for this were not investigated, but the effect occurred in both non-treated and microwave-treated soils, suggesting that root-infecting pathogens or minor pathogens (Salt,1970) could have been involved.

CHAPTER 4

INVESTIGATION OF SPATIAL SUCCESSION OF MYCORRHIZAL FUNGI ON THE ROOTS OF BIRCH SAPLINGS PLANTED IN TROUGHS OF SOIL

4.1. Introduction

As described in Section 1.5, spatial and temporal successions of fruitbodies of mycorrhizal fungi have been recorded in two plots of birch at I.T.E.,Bush Estate (Ford *et al*,1980; Mason *et al*,1982,1983 b, 1984; Last *et al*,1984 a,b),and a broad distinction has been made between early stage and late stage fungi in these successions, depending on the age of the trees on which fruitbodies of the fungi first appeared. The broad distinction was confirmed by observations of the patterns of occurrence of mycorrhizas below ground (Deacon *et al*, 1983) and is supported by other evidence (Fleming,1983 a,b,1984; Fleming *et al*,1984; Fox,1983,1986). Recently, Fleming *et al*(1986) suggested that the distinction, although still valid, can be masked by soil factors.

The spatial aspects of mycorrhizal succession have received little study to date, mainly because experimental analysis of this phenomenon requires the use of specially designed plant growth containers in which parts of the root systems are distributed in different regions of soil - a requirement difficult to achieve in conventional plant pots. The experiments in this section of the thesis involved the development of a suitable system to investigate the phenomenon of spatial succession.

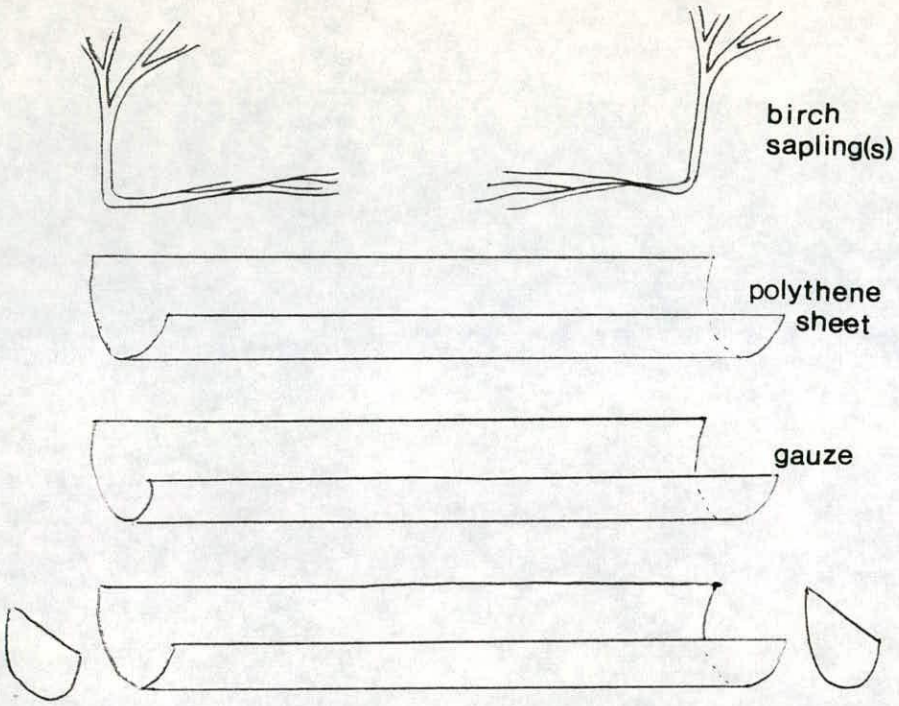
4.2. General Method

4.2.1. Preparation of growth containers.

Plastic drainpipe of 11 cm diameter was cut into 70 cm lengths and then sawn longitudinally to provide semi- circular halves. The ends of each half were fitted with semi-circular pieces of perspex or plastic-covered cardboard which were taped into position such that water could drain from the pipes. The shallow troughs thus formed were lined with pieces of fine nylon gauze (70 x 15 cm), followed by thick polyethelene sheeting (70 x 17 cm) as shown in Fig 4.2.1. In autumn (Experiment 1) or late spring (Experiment 2) naturally regenerating birch saplings, approximately 25 cm tall, were carefully excavated from the extreme edge of a zone of birches colonising a disused coal spoil heap at Newtongrange, Midlothian. The saplings were growing just beyond a zone where the spoil was hot as a result of "after- burning" and were beyond the influence of the roots of larger, established trees at the edge of the spoil heap. Each sapling was transported to the laboratory in a closed polythene bag, its root system was carefully washed and the predominant mycorrhizal types on it were recorded. Only a few mycorrhizal tips were visible and distinguishable and those were mainly attributable to *Scleroderma* spp. and *Paxillus* spp.. Then the saplings were placed singly at one or both ends of the troughs, with the shoot close to the end and the roots aligned longitudinally along the bottom of the trough, above the gauze and polythene linings. The troughs were immediately filled with soil and watered.

The soil was a brown earth from a treeless hill pasture at Castlelaw, south of Edinburgh, with the characteristics shown in the table below.

Fig 4.2.1
Diagram of trough construction and layout in glasshouse.



trough – drainpipe with perspex or cardboard ends

Chemical analysis of brown earth collected from Castlelaw, south of Edinburgh; the results represent the values of two samples, all being expressed on a dry weight basis, $mg.100g^{-1}$ (Fox, 1983).

		Sample 1	Sample 2
	pH	5.2	5.2
	K	20	22
extractable	Ca	330	300
	Mg	20	18
	Fe	35	290
	P	11	9
	P%	0.14	0.17
extractable	$NO_2 + NO_3$	11	24
	NH_4	7.1	0.27

Before use, it was sieved < 10 mm and mixed with washed grit (4:1 v/v). The troughs containing saplings were arranged on racks a few centimetres above the concrete base of a large glasshouse with no supplementary lighting or heating but with automatic, thermostatically controlled fan and vents. Maximum temperatures in summer seldom exceeded 17°C, and the minimum winter temperatures were maintained just above freezing by means of paraffin heaters. Troughs containing two saplings (Experiment 1) were moved to a glasshouse that was heated (minimum temperature 8° C). The troughs were watered as necessary during the course of sapling growth.

4.2.2. Inoculation procedure.

Various types of mycorrhizal inoculum were added to the root zone of the birch saplings during the experiments. To achieve this, a length of boarding was made, with notches in its ends to accommodate the stems of the saplings, and it was placed on the soil surface in a trough. Then the trough was inverted on to the board, the trough was removed and the nylon gauze and the polythene

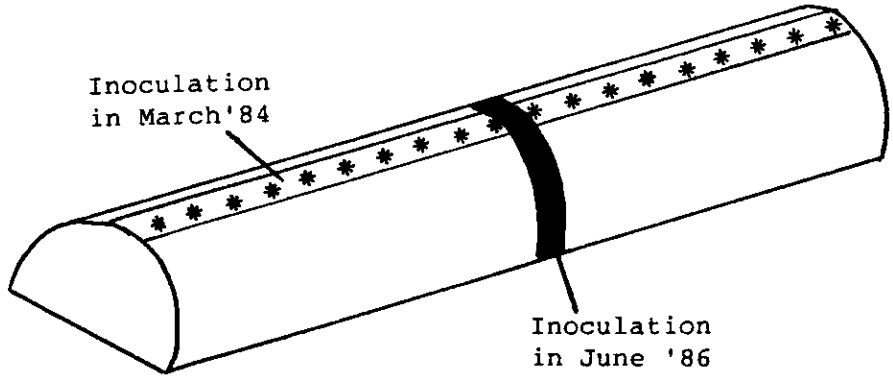
lining were carefully peeled away to expose the soil "core". (Fig 4.2.2). The inoculum, comprising colonised vermiculite-peat or liquid culture (Section 2.2.2) was applied to different parts of the exposed root system , either all the way along the bottom of the "core" or in specific positions that were marked by placing over them narrow strips of nylon gauze. Then the troughs were re-assembled as before and re-incubated on racks. In some troughs a spore inoculum was used; it consisted of basidiospores released from freshly collected fruitbodies and stored as spore prints on paper, in jars at 4°C for 4 months. The spores were removed from the paper, suspended in distilled water and applied by syringe, at selected positions, just below the upper surface of the soil.

Most localised inoculation treatments were applied in "bands" roughly 1 cm wide, and spaced equidistantly along the troughs, the bands being numbered 1 - 7, starting closest to the tree base (Fig 4.2.2). Individual troughs received different inoculation treatments or combinations of treatments in a pre-determined experimental design to ensure adequate replication and randomisation of treatments.

4.2.3. Periodic recording of mycorrhizas.

At intervals during the experiments, the core of soil in each trough was exposed as described in (2) above, to examine the distribution and types of mycorrhizas on the root systems. Two examples of mycorrhizal types visible on the lower surface are shown in Fig 4.2.3. Sometimes individual mycorrhizal tips were removed with forceps for microscopic examination to confirm visual identifications. Records of mycorrhizal distribution were made by laying a sheet of clean polythene film over the soil and marking areas occupied by different mycorrhizal types (Fig 4.2.4), then removing the film before the troughs were

Fig 4.2.2
Positions and types of inocula applied to troughs in Experiment 1.



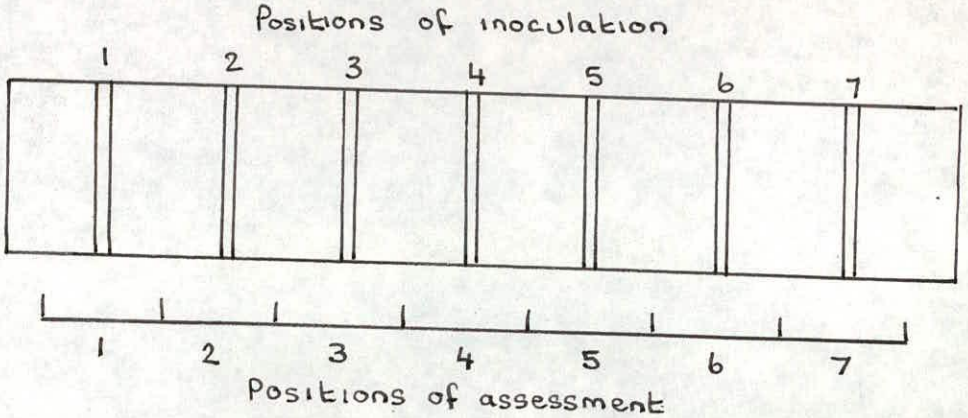
Trough	Inoculation in March'84	Inoculation in June '86
1/2	Hs	-
3/4	Lpub	-
5/6	Lpub	-
7/8	Hs	-
9/10	Control	-
11/12	Control	Lpub
13/14	Control	Lpub
15/16	Hs	Lpub
17/18	Hs	Lpub
19/20	Hs	Lpub
21/22	Control	Lpub
23/24	Control	Lpub
25/26	Lpub	Lpub

(✱, vermiculite-peat inoculum ; , liquid-culture inoculum
Hs = *Hebeloma sacchariolens* isolate 4
Lpub = *Lactarius pubescens* isolate 4
control = sterile v-p applied
- = troughs sampled before June 1986

Fig 4.2.3
Mycorrhizas of a) Lactarius-type and

b) Lactarius-type (top) with patches of Leccinum-type (white) plus attached mycelial strands on lower surface of troughs.

Fig 4.2.4
Example of "map" recording of mycorrhizal types visible on the lower soil surface, and diagram to show position of "bands" in which percentage cover by each visible mycorrhizal type was calculated.



reassembled. The percentage of surface cover by each mycorrhizal type within each sampling "band" (Fig 4.2.4) was calculated by placing the "maps" over a grid and counting the proportion of the squares in each sampling band that was occupied by each type. Any changes in mycorrhizal types or distribution with time could thus be monitored by placing the polythene overlays (or maps) on top of one another in the laboratory. An incidental advantage of this procedure was that the fine roots were not subjected to desiccation during observation.

4.2.4. Recording of fruitbodies.

The positions and types of fungal fruitbodies were recorded as and when they appeared in individual troughs as shown in Fig 4.2.5. The fruitbodies were usually removed for precise identification.

4.2.5. Insertion of seedlings.

Young (8 wk-old) non-mycorrhizal birch seedlings were produced in aseptic culture (Section 2.2.1) and planted in a series of pre-determined positions along a central longitudinal line in each trough, so that the mycorrhizas that developed on them could later be compared with those in similar positions on the larger sapling root system. In addition, similar non- mycorrhizal seedlings were planted along the sides of the troughs (Fig 4.2.6), between the soil and polythene lining, at positions of dense mycorrhizal establishment on the sapling root system, to test for infection of seedling roots from established mycorrhizas. In some instances, young (12 wk-old) seedlings, on which mycorrhizas of known fungi had developed, were inserted between the soil and polythene lining, as described above, to test for transfer of mycorrhizal types between the seedling and sapling root system.

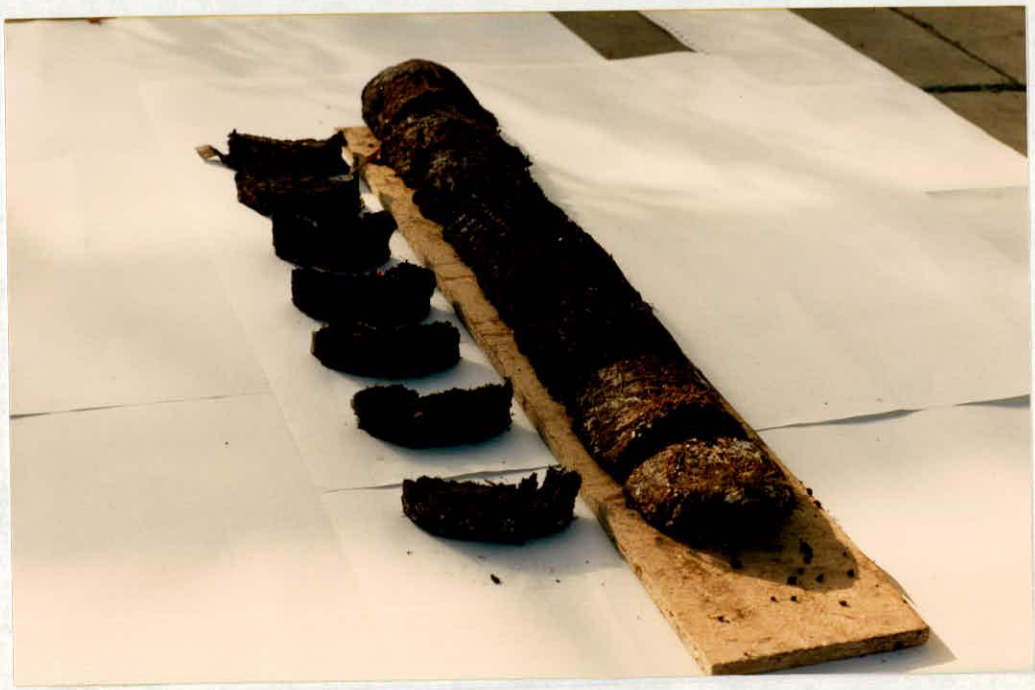
Fig 4.2.5
Production of fruitbodies of *Inocybe* (I), *Hebeloma* (H) and *Thelephora* (T) in troughs containing one birch sapling.

Fig 4.2.6
Illustration of seedlings inserted down the sides of troughs.

4.2.6. Sampling procedure.

On sampling of the troughs,the shoots were excised at soil level and the stem base sectioned to determine the age of the plant. The troughs were overturned on a piece of board and the plastic trough, gauze and polythene were removed (Fig 4.2.4). Seedlings that had been inserted between the soil and the polythene lining were carefully removed for examination. Then 1 cm wide "slices" of soil were cut at regular intervals (usually 9 cm) along the soil cores (Fig 4.2.7) and also in positions where dense mycorrhizal development was seen. The soil slices were placed on a sieve, washed under running tap water and the root fragments were then spread in water in a Petri dish for examination under a stereoscopic microscope. The total number of root tips in each 1 cm section of soil and the number attributable to different mycorrhizal types were recorded. The procedure described above was the one that was finally adopted, but in some early samplings an attempt was made to dissect out a representative sample of main, longitudinally running structural roots and their fascicles of attached branches and fine roots. Also in some earlier samplings, cores of soil, 1 cm diameter, were removed with a cork borer inserted vertically into the troughs, so that the distribution of mycorrhizas in relation to soil depth could be determined.

Fig 4.2.7
Position and appearance of sample 1 cm-wide "slices" (marked by gauze strips) taken at 9 cm intervals along the length of the sapling root system.



4.3. Experiment 1

4.3.1. Method.

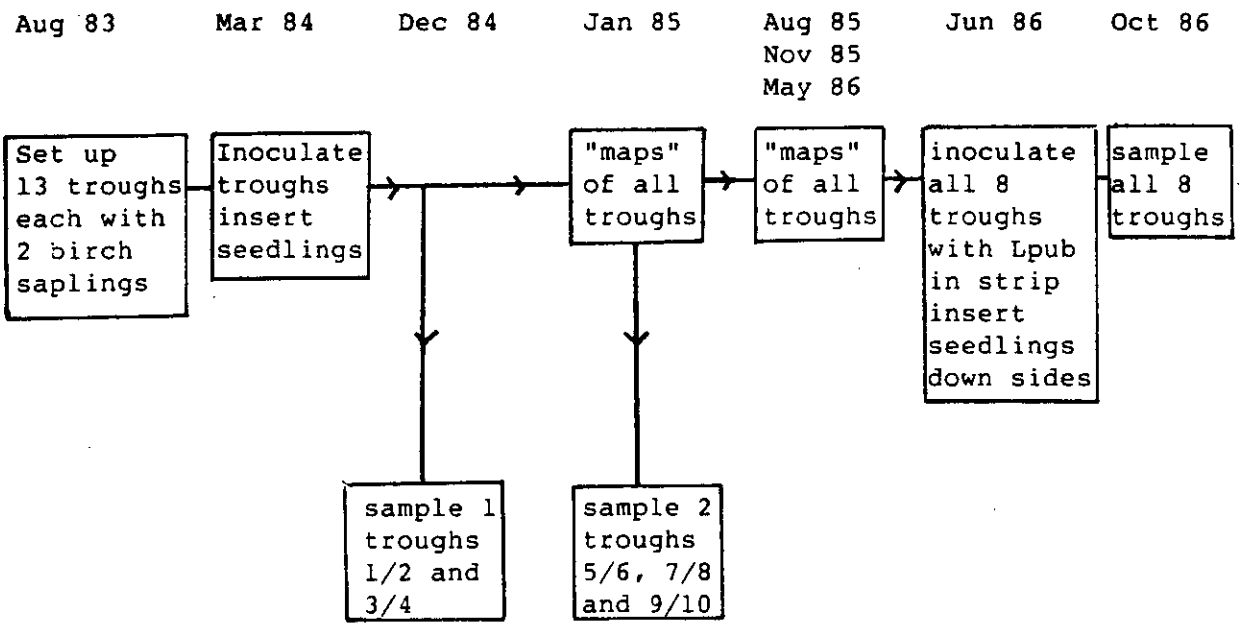
Thirteen shallow troughs were prepared in autumn 1983 as described in Section 4.2.1. Two birch saplings, excavated from a disused coal spoil heap at Newtongrange, Midlothian, were placed in each trough, one at each end, and the trough filled with a "non-mycorrhizal" brown earth (Section 4.2.1).

The troughs were arranged on racks and placed in a glasshouse with supplementary heating (to provide a minimum of approximately 8°C) and lighting (12 h daylength) for 4 weeks to encourage root growth. Then bud formation was promoted by transferring the troughs to a large growth cabinet which provided a warm environment (19° C day mean, 13°C night mean) with an 8 h light,16 h dark cycle for a period of 6 weeks. After this time the troughs were transferred to a large unheated glasshouse (where dormancy was imposed on the saplings) with no supplementary lighting but which was kept frost-free by means of a paraffin heater in winter and was cooled in summer by thermostatically controlled vents and a fan. The troughs remained in this glasshouse for the duration of the experiment.

At the onset of root extension in the following spring the troughs were inoculated as shown in Fig 4.3.1 with a "bottom layer" of vermiculite-peat colonised by either *Hebeloma sacchariolens* or *Lactarius pubescens*, with sterile vermiculite- peat in some instances for control troughs (Section 4.2.2); seven non- mycorrhizal birch seedlings were inserted at intervals along the length of each trough at this time (Section 4.2.5).

After incubation for 9 months, five troughs were destructively sampled

Fig 4.3.1
Flowchart showing experimental procedure for Experiment 1.



(Section 4.2.6) - as shown in the flowchart in Fig 4.3.1 - by attempting to dissect out a representative sample of five main longitudinal roots, with attached branches and fine roots, from each tree. The roots were washed carefully and cut into 5 cm numbered sections starting from the tree base. Initially, for two troughs only, the presence or absence of each mycorrhizal type, assessed microscopically, was recorded for each section, but later (for three further troughs) the numbers of mycorrhizal tips attributable to each fungus were counted in each section. Also, cores of soil, 1 cm diameter, were taken through the depth of the soil in each trough with a cork borer to determine the depth-distribution of roots in the soil.

In June 1986 each of the remaining eight troughs was inoculated with liquid culture inoculum of *Lactarius pubescens* in a 1 cm wide band in a central position marked by a strip of gauze. Then 28 birch seedlings (12 wk old), mycorrhizal with *Hebeloma* or *Thelephora* or non-mycorrhizal , were inserted down the sides of the troughs as described in Section 4.2.5.

The troughs were watered as necessary throughout the experiment and recordings of the mycorrhizas visible on the lower surface of the troughs (Section 4.2.3) were made periodically. Fruitbodies appearing on the troughs were identified, and their positions were recorded prior to removal (Section 4.2.4).

In October 1986, the remaining troughs were destructively sampled, as described in Section 4.2.6, and the roots in seven one-cm wide "slices" of soil taken equidistantly along each trough (Fig 4.2.7) were carefully washed out and examined microscopically. Where possible, seedlings also were removed and their roots were assessed.

4.3.2. Results - Sample 1.

Two troughs were sampled in January 1985 (troughs designated 1/2 and 3/4 - these being the numbers used to designate individual saplings) and were assessed microscopically according to the presence of different mycorrhizal types obvserved in 5 cm sections of five main roots from each tree. Only two mycorrhizal types were observed - those attributable to *Hebeloma* and *Inocybe* - and their distributions are shown in Figs 4.3.2 and 4.3.3, which show also the relative positions of the root regions assessed for the two trees in each trough.

This preliminary assessment revealed differences in occurrence of mycorrhizas both along the lengths of the troughs and between the two trees in each trough. In particular, mycorrhizas of *Hebeloma* occurred predominantly at one end of the first trough (Fig 4.3.2) - on the older part of the root system of tree 1 and the youngest part of the root system of tree 2 - while in the second trough (Fig 4.3.3) only one of the two trees (designated "3") bore mycorrhizas of *Inocybe*, and tree 4 bore mycorrhizas only of *Hebeloma*. This result was interesting because the root systems of the two trees in each trough overlapped and were in close contact in some regions. So, either the trees differed in their receptivity to development of different mycorrhizal types such as *Inocybe*, or these mycorrhizal types developed mainly from pre-existing mycorrhizas on the different trees and there was little ability of *Inocybe* to spread from established mycorrhizas on one tree to the root system of another tree in close proximity.

The results of visual assessment of mycorrhizal development by *Hebeloma* on the lower surface of the two troughs before they were sampled are shown in Figs 4.3.2 and 4.3.3 . The observations correspond in general to those of the microscopical assessments, by showing, in particular, a predominance of

Fig 4.3.2
Sample 1.
Distributions of mycorrhizal types along the length of roots of two birch saplings planted at opposite ends of a trough of soil (at top and bottom of figure) based on microscopical examination of 5 cm sections of 5 roots of each tree ; the central figure shows the distribution of visible mycorrhizas on the lower surface of the trough at the time of sampling.

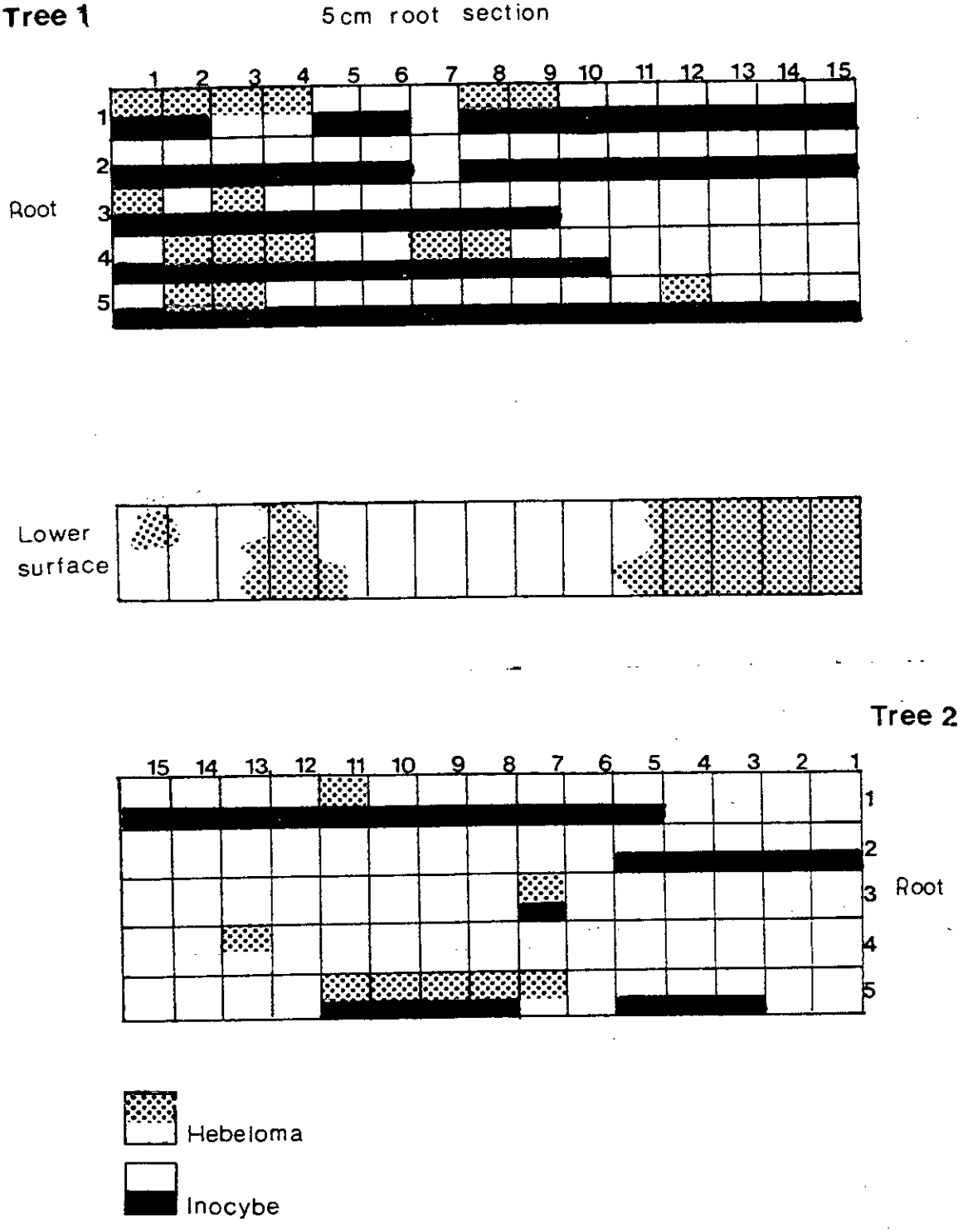
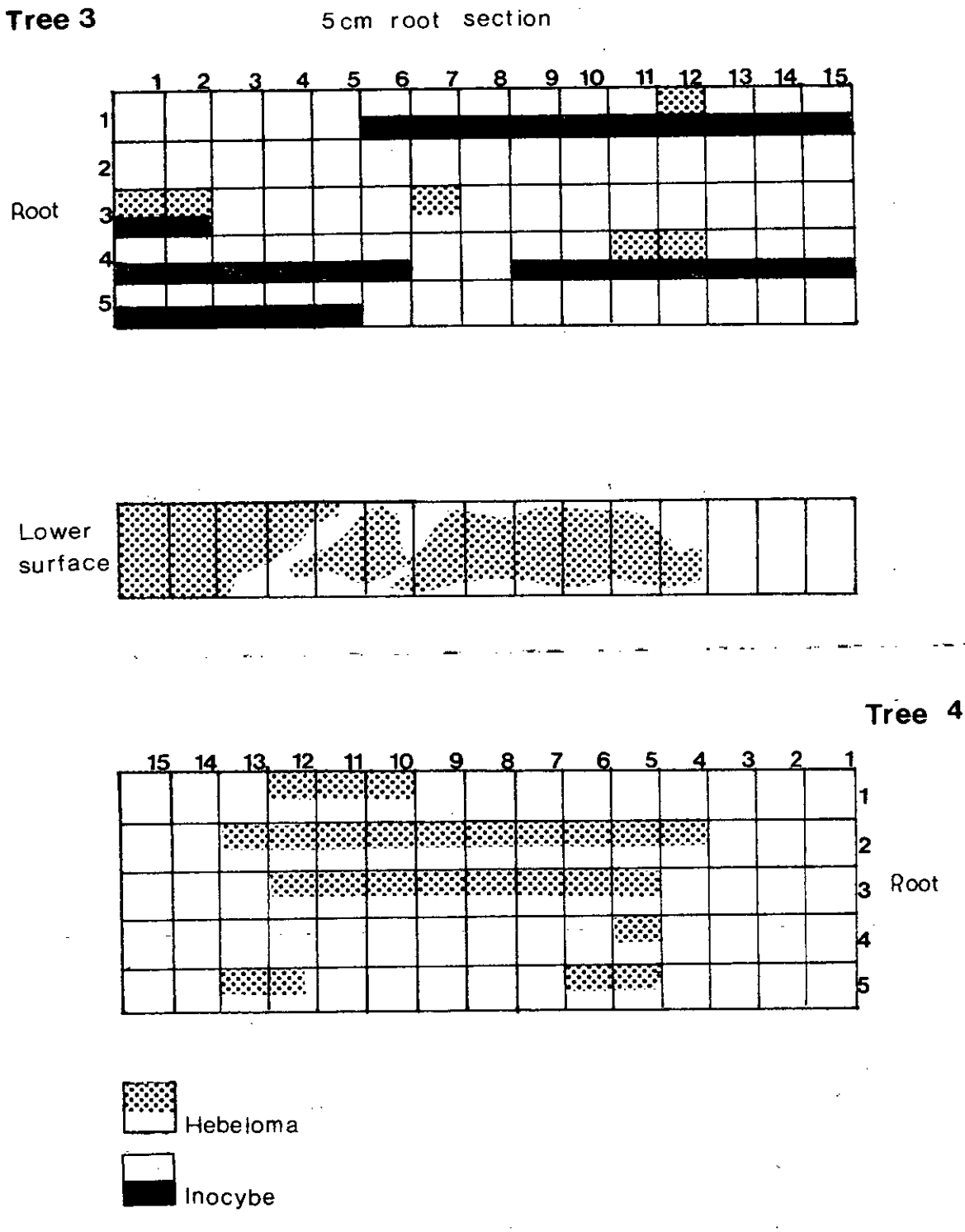


Fig 4.3.3. (Legend as for Fig 4.3.2)



this mycorrhizal type at one end and in the middle region of the two troughs.

4.3.3. Results – Sample 2

Three further troughs (5/6, 7/8 and 9/10) were sampled in January 1985, and the percentage occurrence of different mycorrhizal types was assessed in 5 cm root sections (Figs 4.3.4 – 4.3.6). All three troughs contained mycorrhizas of *Inocybe* and *Hebeloma* ; one trough (7/8) also contained mycorrrhizas of *Thelephora* and another trough (9/10) contained mycorrhizas of *Cenococcum*. Prior to destructive sampling, the distribution of *Hebeloma* mycorrhizas visible to the naked eye on the lower surface of each trough was recorded and the percentage of surface cover by this mycorrhizal type is also shown in Figs 4.3.4 – 4.3.6.

The results of these assessments show considerable variability between the three troughs, although mycorrhizas of *Inocybe* were predominant in all three and were more or less generally distributed along the length of the root systems, except on tree 6 (Fig 4.3.4). Mycorrhizas of *Hebeloma* were generally distributed along the roots of trees 9 and 10 (Fig 4.3.6) with no obvious difference in establishment on old compared with young regions of the root systems. In this trough visual inspection suggested that Hebeloma-type mycorrhizas predominated in the centre region of the trough, but this was not confirmed by microscopic observations. In trough 7/8 (Fig 4.3.5) visual observations suggested that Hebeloma-type mycorrhizas predominated at the ends rather than in the central region – a result supported by microscopic observations. In trough 5/6 (Fig 4.3.4) the distribution of Hebeloma-type mycorrhizas was very variable but, in general, the distribution assessed microscopically was supported by visual observation of the soil surface. Except

FIG 434

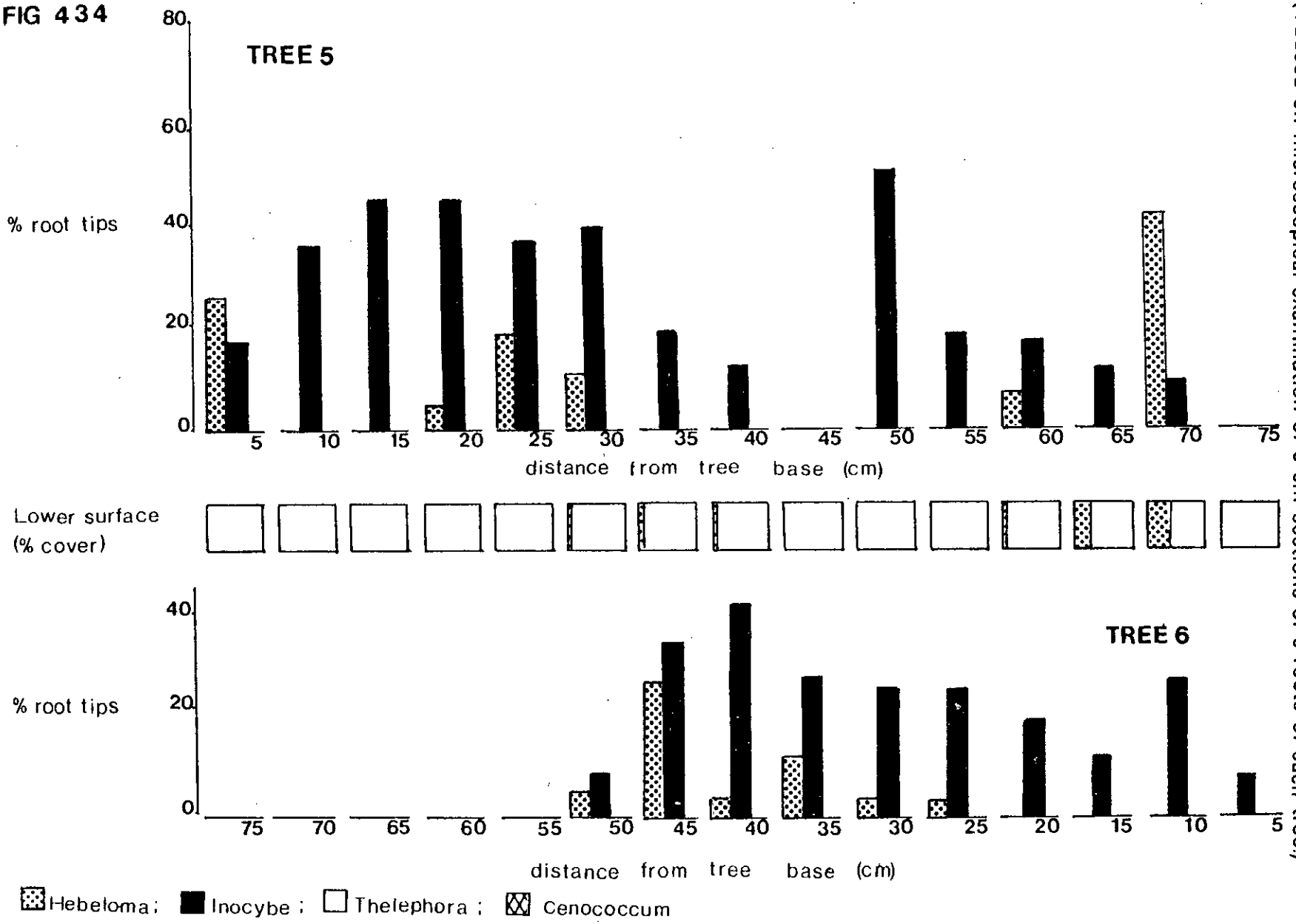


Fig 4.3.4
Percentage of root tips attributable to different mycorrhizal fungi* and the percentage cover of the lower soil surface by identifiable mycorrhizal types (centre) in troughs containing 2 birch saplings planted at opposite ends of troughs of soil.
(*based on microscopical examination of 5 cm sections of 5 roots of each tree.)

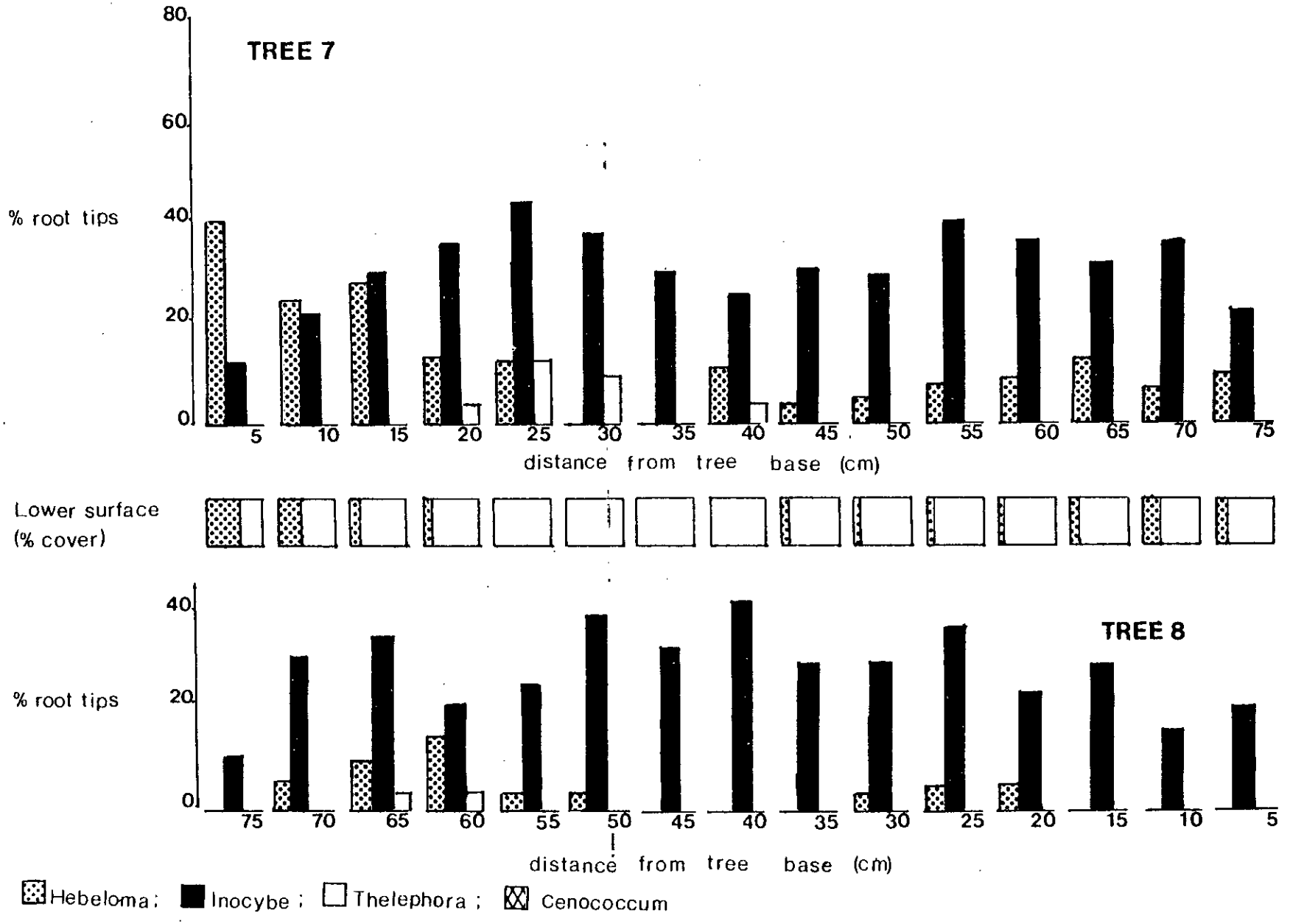


Fig 4.3.5 (Legend as for Fig 4.3.4)

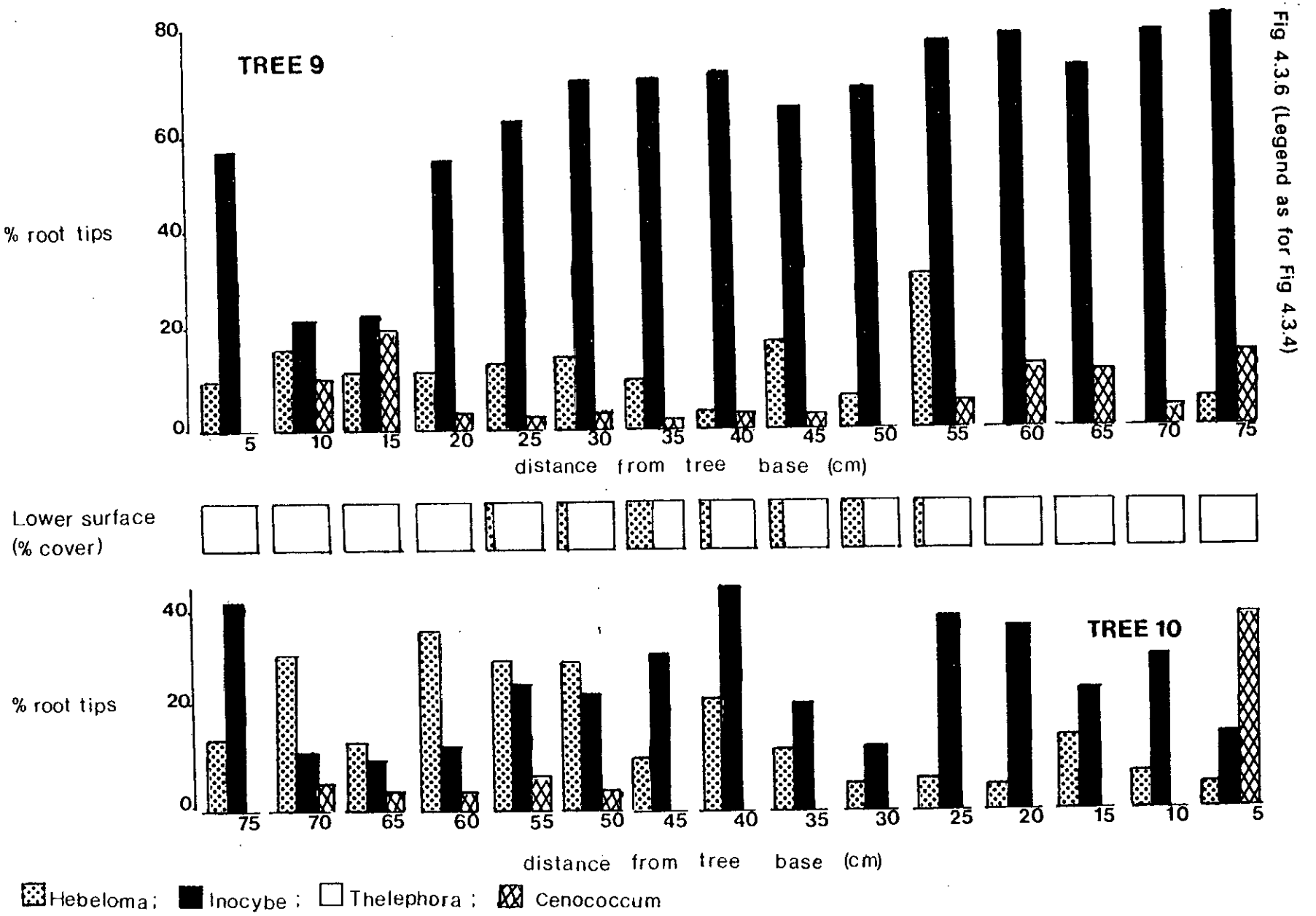


Fig 4.3.6 (Legend as for Fig 4.3.4)

in trough 5/6,there was evidence that Hebeloma-type mycorrhizas and also those of *Cenococcum* and *Thelephora* occurred in corresponding positions on the overlapping roots of the two trees in any one trough. This suggests that cross-inoculation may have occurred or that environmental conditions were most suitable for development of the particular mycorrhizal types in specific regions of the troughs, but it gives little indication that the age of a region of a root system influenced the success of mycorrhizal development by the fungi that occurred in this experiment.

The results of soil coring in the troughs are summarised below and indicate clearly that most root tips occurred in the bottom 1 cm of soil.

Distance from upper soil surface(cm)	Percentage of total root tips
0.0 - 4.0	5
4.1 - 5.0	16
5.1 - 5.5	32
5.6 - 6.0	47

4.3.4. Results - Sample 3.

In October 1986 the remaining eight troughs were sampled by cutting seven "slices" of soil, each 1 cm wide, from positions along each trough. The soil slices and the seedlings that had been inserted down the sides of the troughs were prepared and assessed as described in Section 4.2.6. It proved impossible to remove the surviving seedlings that had been inserted into central positions in the troughs in 1984, because the seedling roots had intermingled with the older tree roots and could not be dissected from these.

Distribution of root tips. The distribution of root tips along the troughs and the degree to which these root tips were mycorrhizal with different

fungi are shown in Tables 4.3.1 - 4.3.9. Somewhat more than 1000 root tips were counted, on average, in each of the seven sampling positions in each trough but there were significantly (P=0.05) more root tips in the central position than in all others when the results were subjected to analysis of variance (Table 4.3.1). This might reflect the fact that the root system of each tree attained maximum root density about 35 cm from the tree base or it might have occurred in response to the addition of inoculum of *Lactarius* in the central position if a substantial amount of nutrients was present in the liquid inoculum applied in June 1986. The percentage of root tips that were mycorrhizal (Table 4.3.2) differed markedly between troughs and to a lesser degree between sampling positions within troughs. Of interest, there was no significant difference in mycorrhizal development between the central sampling position (number 4) and other positions, although the total number of mycorrhizas in the central position was slightly higher than elsewhere, reflecting the larger number of root tips in general in this position.

Effects of inoculation on mycorrhizal development The patterns of inoculation of the eight troughs sampled in October 1985 are shown in Fig 4.2.2 and the percentages of root tips attributable to different mycorrhizal types in the troughs are shown in Tables 4.3.3 - 4.3.9. Inoculum of *H. sacchariolens* had been added along the length of troughs 15/16, 17/18, and 19/20 in March 1984. These three troughs subsequently developed Hebeloma-type mycorrhizas along their lengths (although these mycorrhizas were not neccessarily attributable to *H.sacchariolens per se*), but a comparison of the mean occurrence of Hebeloma-type mycorrhizas in the 21 sampling positions of these troughs (7.14 + 1.19) and in the corresponding 28 positions in the 4 uninoculated troughs (5.69 + 0.79) revealed no significant effect of inoculation (see Table 4.3.4). Inoculum of *L.pubescens* was added along the length of one trough (25/26) in March 1984 and

Tables 4.3.1 - 4.3.9
Experiment 1. Growth and mycorrhizal development of roots of birch saplings grown in troughs (see text for details) and sampled in October 1986.

Table 4.3.1
Total number of root tips per sampling position

Trough	Sampling position						
	1	2	3	4	5	6	7
11/12	2789	1819	1808	2956	1211	927	1181
13/14	506	1268	3175	3695	1863	1563	1039
15/16	981	1573	1064	3298	794	1037	1526
17/18	1280	1406	2359	1390	2012	2548	1068
19/20	626	1032	398	2966	1752	1052	3376
21/22	1348	853	374	1965	1237	1137	264
23/24	1818	1218	1134	2248	705	404	786
25/26	1395	1008	570	2133	1229	240	290
Mean	1343	1272	1360	2581	1350	1113	1192
		SED	(608)				
		5%LSD	(1229)				

Table 4.3.2
Percentage mycorrhizal root tips per sampling position

Trough	Sampling position						
	1	2	3	4	5	6	7
11/12	18.2	31.5	27.8	34.0	32.4	40.5	33.8
13/14	65.1	51.0	55.7	55.4	49.6	59.2	66.6
15/16	51.8	29.4	25.1	37.8	46.6	22.9	28.1
17/18	42.4	55.9	48.0	64.7	51.5	56.0	36.1
19/20	60.6	29.9	38.4	56.6	26.6	39.6	42.3
21/22	44.5	55.5	71.4	40.8	40.2	40.0	33.3
23/24	36.6	43.7	51.2	63.0	23.7	39.6	56.2
25/26	41.8	53.9	49.8	58.0	45.8	43.3	71.7
Mean	45.1	43.9	45.9	51.3	39.6	42.6	46.0
		SED	(7.1)				
		5%LSD	-				

Table 4.3.3
Percentage tips attributable to Laccaria *

Trough	Sampling position						
	1	2	3	4	5	6	7
11/12	0	0	0	0	0	0	0
13/14	0	0	0	0	0	0	0
15/16	0	0	0	0	0	0	0
17/18	0	0	0	0	0	0.6	0
19/20	0	0	0	0	0	0	0
21/22	0	0.3	1.3	0	0.3	1.4	0
23/34	0	0	0	0	0	0	0
25/26	3.3	0	0	2.7	0	0	8.3

* too few observations to enable statistical analysis.

Table 4.3.4
Percentage root tips attributable to Hebeloma

Trough	Sampling position						
	1	2	3	4	5	6	7
11/12	4.7	5.7	2.4	6.4	5.8	7.0	4.0
13/14	5.7	12.8	7.0	9.6	9.5	9.4	11.6
15/16	3.3	5.6	4.7	2.8	6.9	4.3	6.7
17/18	10.8	8.5	17.6	21.2	15.9	10.5	2.9
19/20	10.5	2.9	4.5	2.7	2.4	2.2	3.1
21/22	8.3	8.4	16.0	2.7	6.1	4.9	8.3
23/24	0.9	0	0	1.4	0	0.9	0
25/26	11.1	3.2	0	0.7	0	0	5.9
mean	6.9	5.9	6.5	5.9	5.8	4.9	5.3

SED (3.4)
5%LSD -

Table 4.3.5
Percentage root tips attributable to Lactarius

Trough	Sampling position						
	1	2	3	4	5	6	7
11/12	0	0	0	0	0	0	0
13/14	12.8	12.8	3.2	10.9	7.5	16.6	12.3
15/16	42.8	16.8	9.1	6.3	31.3	15.9	16.6
17/18	2.3	11.7	0	0	1.8	0	0
19/20	35.0	20.1	29.4	9.7	0	0.8	5.8
21/22	12.1	5.1	4.5	2.5	5.6	10.9	0
23/24	4.8	4.6	3.5	1.3	2.5	7.7	2.4
25/26	3.9	20.9	3.1	8.1	22.8	13.3	11.0
mean	14.2	11.5	6.6	4.8	8.3	8.2	6.0

SED (4.87)
5%LSD -

Table 4.3.6
Percentage root tips attributable to Thelephora

Trough	Sampling position						
	1	2	3	4	5	6	7
11/12	9.8	10.4	3.3	7.2	11.9	6.2	4.5
13/14	12.2	0.9	15.3	7.1	3.5	4.3	9.9
15/16	3.0	2.3	3.6	7.9	6.3	0	0
17/18	16.2	16.8	21.5	14.7	15.5	11.6	13.8
19/20	0	2.7	0	4.9	7.1	10.8	10.8
21/22	23.1	23.2	30.5	12.8	22.5	16.5	17.0
23/24	30.1	20.8	19.2	25.5	0	11.9	21.9
25/26	23.9	18.1	26.5	22.7	10.0	15.4	23.5
mean	14.78	11.9	14.99	12.85	9.6	9.6	11.4

SED (4.40)
5%LSD -

Table 4.3.7
Percentage root tips attributable to Inocybe

Trough	Sampling position						
	1	2	3	4	5	6	7
11/12	7.4	5.7	3.9	5.2	8.9	16.4	9.0
13/14	20.4	19.6	26.3	10.7	11.2	22.4	12.8
15/16	9.5	4.1	7.7	7.0	2.3	2.6	2.4
17/18	12.4	18.2	7.5	18.8	16.3	20.0	15.3
19/20	28.8	3.7	0	18.4	7.9	11.6	16.5
21/22	6.5	18.3	19.0	15.8	4.0	4.0	7.9
23/24	9.2	12.5	22.0	16.0	20.4	16.1	24.3
25/26	20.8	2.9	8.8	14.8	13.0	5.0	23.1
mean	14.4	10.6	11.9	13.3	10.5	12.3	13.9

SED (3.2)
5%LSD -

Table 4.3.8
Percentage root tips attributable to Cenococcum

Trough	Sampling position						
	1	2	3	4	5	6	7
11/12	10.2	11.8	17.9	15.2	1.2	1.8	0.8
13/14	23.7	4.7	3.8	17.0	18.5	6.4	19.9
15/16	0	0	0	0	0	0	0
17/18	0.6	0.6	1.4	8.0	1.8	13.2	4.1
19/20	3.3	0.5	4.5	0.9	10.7	13.9	5.6
21/22	4.5	0	0	0	1.7	2.6	0
23/24	0.4	0.4	4.3	1.9	0.7	3.0	4.6
25/26	1.5	2.8	1.4	1.1	0	9.6	0
mean	5.5	2.6	4.2	5.5	4.3	6.3	4.9

SED (3.2)
5%LSD -

Table 4.3.9
Percentage root tips attributable to Paxillus *

Trough	Sampling position						
	1	2	3	4	5	6	7
11/12	5.2	7.0	4.9	0	0	0	0
13/14	0	0	0	0	0	0	0
15/16	3.3	0.4	0	3.8	0	0	2.4
17/18	0	0	0	0	0	0	0
19/20	0	0	0	0	0	0	0
21/22	0	0	0	0	0	0	0
23/24	0	0	0	1.1	0	0	0
25/26	0	0	0	0	0	0	0

* too few observations to enable statistical analysis.

then to the central position (4) of each trough in June 1986. Again, however, there was no significant difference between the occurrence of Lactarius-type mycorrhizas in the total 14 positions thus inoculated (mean 6.77 ± 2.03)and the occurrence of Lactarius-type mycorrhizas in other sampling positions (mean 8.53 ± 1.61). On the basis of these results, it seems that both Hebeloma- and Lactarius-type mycorrhizas developed irrespective of inoculation but to varying degrees in each trough for reasons that are unknown.

Development of non-inoculant mycorrhizal types Root tips attributable to *Thelephora* (Table 4.3.6) and *Inocybe* (Table 4.3.7) were observed in , respectively, 51 and 55 of the 56 sampling positions ,and accounted for 12.3 and 12.4 % of all root tips in the 8 troughs. The mean percentage occurrence of each of these mycorrhizal types at the end of the experiment was very similar to that found after 8 months in the previous sampling of three troughs. Neither of these mycorrhizal types was affected by the application of inoculum of *Hebeloma* or *Lactarius* spp.

Mycorrhizas of *Cenococcum* (Table 4.3.8) were observed in all but one trough (15/16) and accounted for a mean 4.7% of the root tips overall. In nine sampling positions *Cenococcum* was the major mycorrhizal type, accounting for up to 23.7% of root tips within a sampling position.

Paxillus-type and Laccaria-type mycorrhizas (Tables 4.3.9 and 4.3.3) were each observed in 8 sampling positions of the 56 , and neither was ever the major mycorrhizal type.

Occurrences of mycorrhizal types visible to the naked eye on the soil surface. The distribution of mycorrhizas visible to the naked eye on the bottom of the troughs was recorded periodically as described in Section 4.2.3. The

observed patterns on each of five occasions are shown in Figs 4.3.7 - 4.3.14.

The mycorrhizal types that were observed and could be identified belonged mainly to *Hebeloma* and *Lactarius,* with one occurrence each of *Leccinum* and *Paxillus* (in troughs 11/12 and 23/24 respectively). Because of its distinctive appearance the recording of Hebeloma-type on the surface is probably the most reliable, but further discussion of this point will be deferred until later (Section 4.4.3). The degree to which the soil surface was covered with obvious mycorrhizal development varied substantially from trough to trough and also between different times for individual troughs. The positions in which each mycorrhizal type developed also varied markedly between troughs and assessment times, such that no general pattern was evident in the experiment as a whole. Nevertheless, the observed patterns of occurrence at the final assessment time (October 1986) can usefully be compared with the numbers of root tips attributable to different mycorrhizal types in the microscopical examination of soil slices that was made immediately afterwards. So the percentage cover of the lower surface by mycorrhizas of *Hebeloma* and *Lactarius* has been calculated for each of seven bands (Tables 4.3.10 and 4.3.11) the mid-points of which correspond to the positions from which soil slices were taken to obtain mycorrhizal counts as shown in Tables 4.3.4 and 4.3.5.

Hebeloma-type mycorrhizas were observed on the soil surface in 33 of the 56 sampling bands for October 1986 (Table 4.3.10). In all of these positions mycorrhizas of *Hebeloma* were also seen in soil slices (Table 4.3.4), with a mean percentage occurrence of 7.4% $\pm$ 0.8. This was significantly (P=0.01) greater than in the 23 positions in which Hebeloma-type was not observed on the soil surface (mean 3.8% $\pm$ 0.8). In fact, of these 23 positions, 7 contained no Hebeloma-type mycorrhizas on microscopic examination of soil slices, 13 had

Table 4.3.10
Percentage of lower surface of troughs covered by Hebeloma-type mycorrhizas.

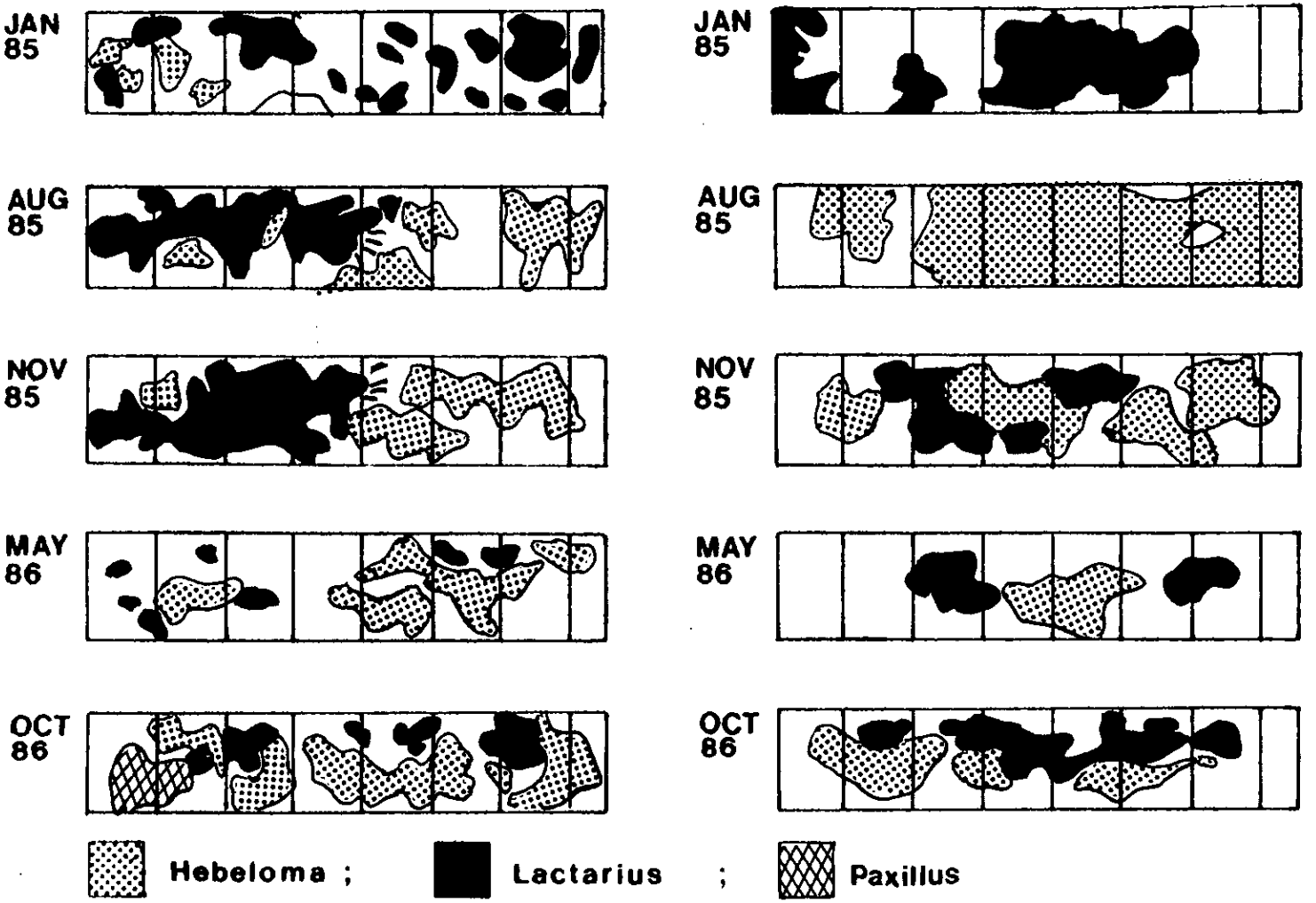
Trough	Sampling position						
	1	2	3	4	5	6	7
11/12	13.7	34.9	9.1	24.6	26.6	15.1	12.6
13/14	66.0	12.3	2.7	14.2	25.0	0	0
15/16	6.6	12.3	2.6	4.3	36.0	0	0
17/18	26.0	35.1	61.7	74.6	30.8	16.9	14.3
19/20	0	0	0	7.4	0	27.7	91.7
21/22	9.4	0.1	8.6	0	14.8	7.1	0
23/24	0	0	0	0	0	0	0
25/26	0	0	0	3.4	0	0	0

Table 4.3.11
Percentage of lower surface of troughs covered by Lactarius-type mycorrhizas.

Trough	Sampling position						
	1	2	3	4	5	6	7
11/12	16.6	14.0	28.9	3.7	27.4	9.4	0
13/14	0	34.6	22.0	25.7	3.7	0	0
15/16	52.3	67.1	64.6	29.4	39.7	86.0	16.8
17/18	0	37.1	16.6	6.3	10.8	25.1	3.7
19/20	75.1	7.4	37.4	0	0	0	0
21/22	25.4	28.9	2.3	18.0	17.1	2.3	17.7
23/24	38.8	40.3	10.8	13.7	18.0	36.6	6.6
25/26	51.1	10.8	21.4	40.6	29.4	31.7	0

FIG 4 3 7 TROUGH 11/12

FIG 4 3 8 TROUGH 13/14



Figs 4.3.7 - 4.3.14
"Maps" of distribution of mycorrhizal types visible on the lower surface of troughs 11/12 to 25/26 each containing two birch saplings.

FIG 439 TROUGH 15/16

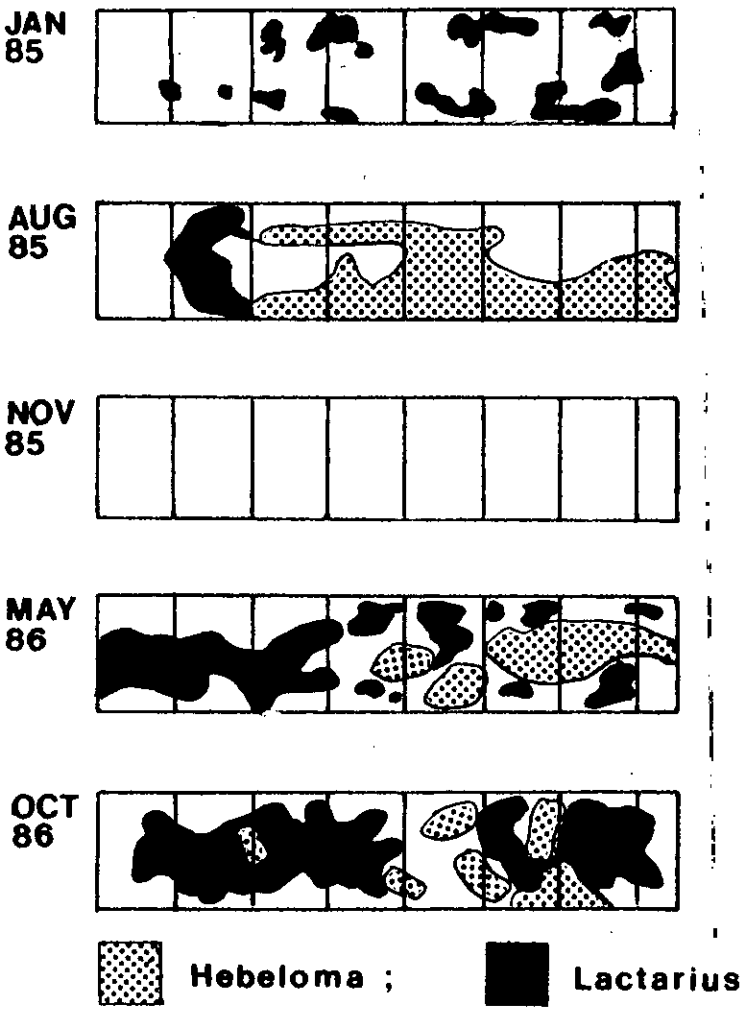


FIG 4 3 10 TROUGH 17/18

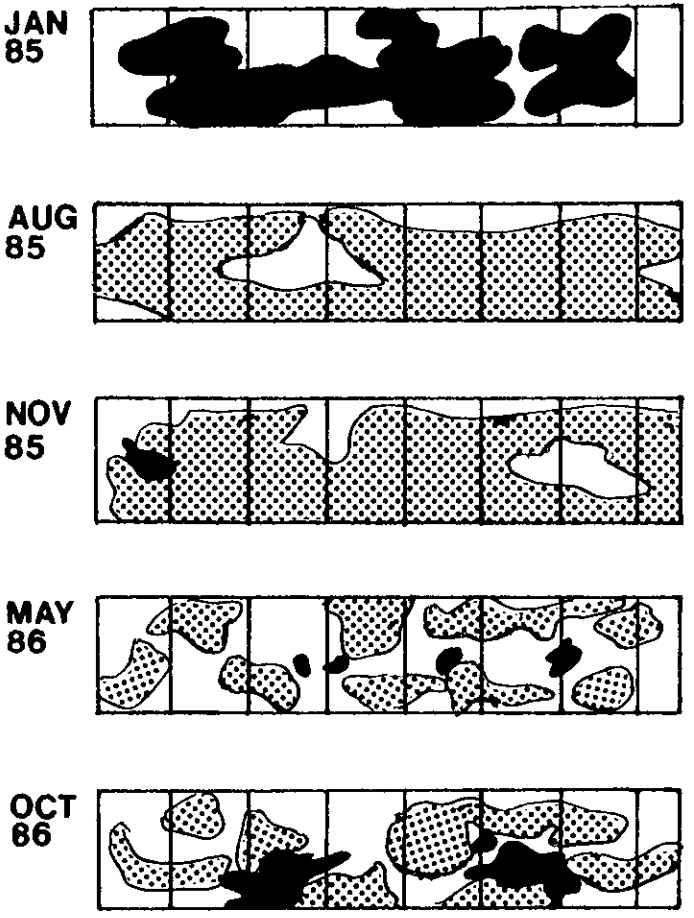


FIG 4 3 11 TROUGH 19/20

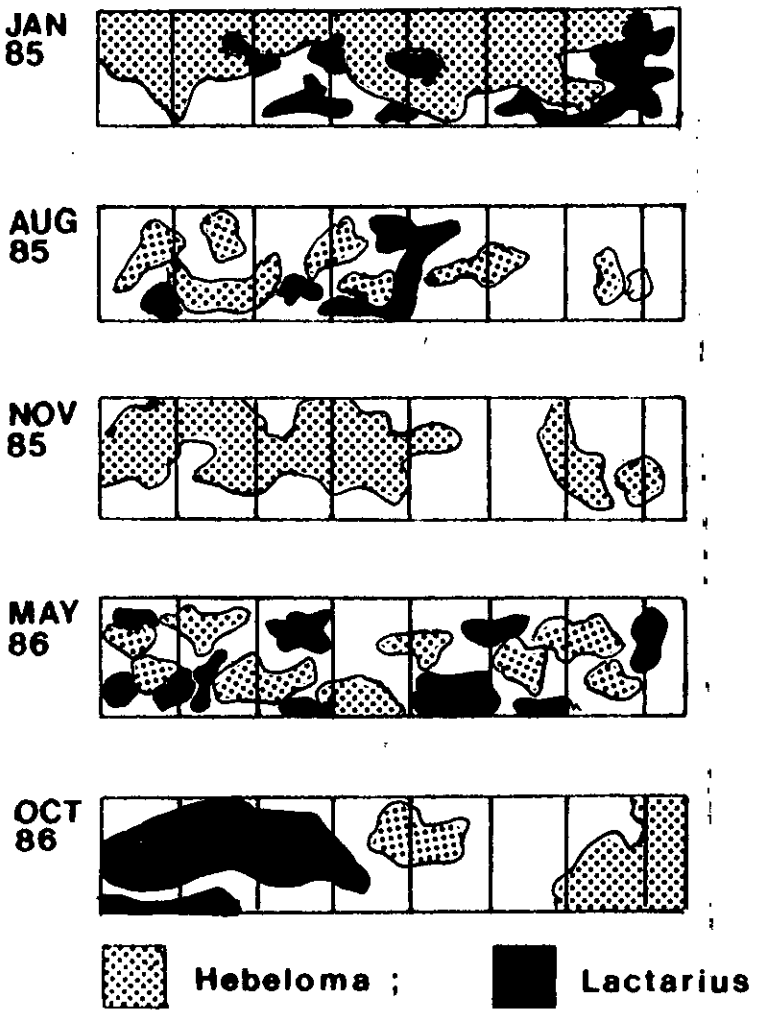


FIG 4 3 12 TROUGH 21/22

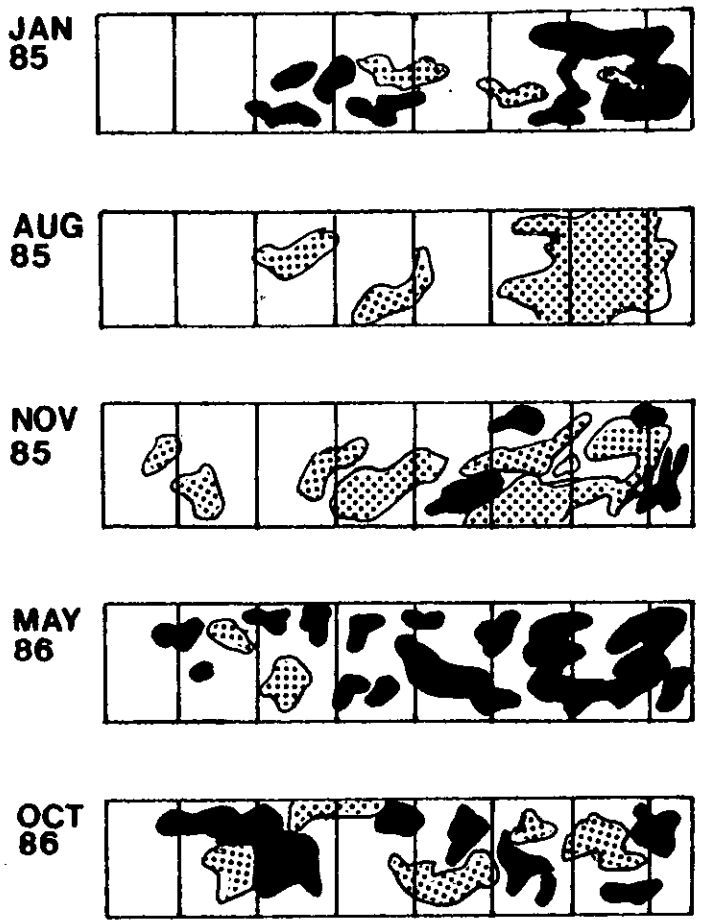


FIG 4 3 13 TROUGH 23/24

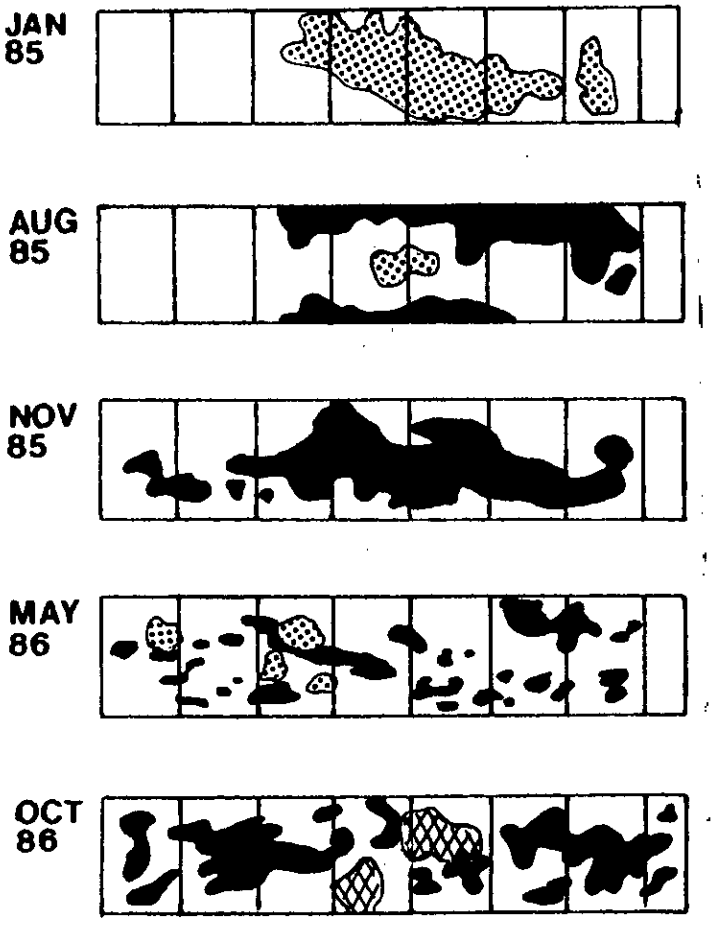
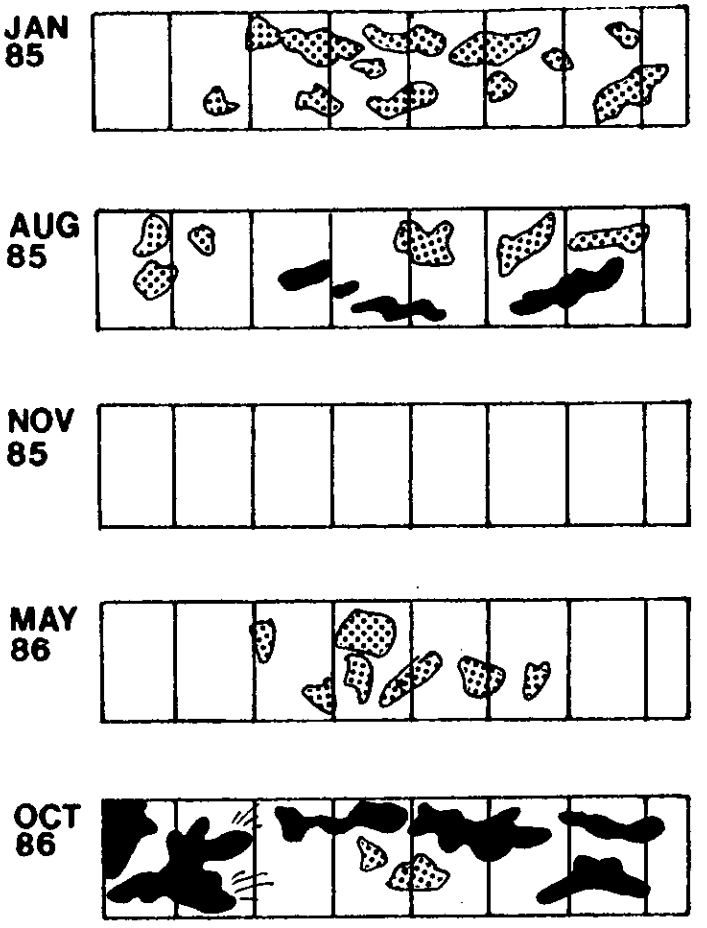


FIG 4 3 14 TROUGH 25/26



Hebeloma ; Lactarius ; Leccinum

less than 10% of root tips attributable to Hebeloma-type mycorrhizas and only 3 had more than 10%. So there was good correspondence between the distribution of Hebeloma-type observed on the soil surface in October 1986 and the numbers of tips attributable to *Hebeloma* in the microscopical examination.

Lactarius -type mycorrhizas were observed on the soil surface in 47 of the 56 positions in October 1986 (Table 4.3.11) but mycorrhizas attributable to *Lactarius* were recorded microscopically in only 35 of the equivalent soil slices, suggesting that mycorrhizas of *Lactarius* were sometimes confused with those of other fungi when examined with the naked eye. Also, in five positions mycorrhizas of *Lactarius* were not observed on the soil surface, although there was an average of 9.3% of root tips attributable to *Lactarius* in corresponding soil slices. This problem will be considered later, in Section 4.4.3.

Development of fruitbodies The types and numbers of fruitbodies that occurred in each trough are shown in Table 4.3.12. The data show firstly that the number of fruitbodies decreased markedly and significantly from 1984 to 1985, and tended to decrease further between 1985 and 1986. These decreases were due primarily to failure of *Inocybe*, *Thelephora* and *Laccaria* to fruit abundantly, if at all, after the first year, although many mycorrhizas of *Inocybe* and *Thelephora* were present in October 1986, when the experiment was ended. A second point revealed by the data is that the occurrence of fruitbodies in different troughs in 1986 bore no relationship to frequency of occurrence of the respective mycorrhizal types in the troughs. For example, fruitbodies of *Hebeloma* occurred only in troughs 19/20 and 21/22 in 1986, but not in trough 17/18 which had many more mycorrhizal tips attributable to *Hebeloma* than in the troughs containing *Hebeloma* fruitbodies. Similarly, *Inocybe* fruitbodies occurred in troughs 17/18, 21/22 and 23/24 in 1986, but not in trough 13/14 which had a

Table 4.3.12
Experiment 1 - Fruitbody summary 1984-86

1984 Trough	Inoc	Thel	Inoc	Heb	Total
11/12	14	0	0	0	14
13/14	0	3	4	0	7
15/16	3	4	3	5	15
17/18	22	4	1	0	27
19/20	6	0	0	0	6
21/22	11	0	1	2	14
23/24	0	0	0	0	0
25/26	5	1	0	0	6
Total	61	12	9	7	89

1985 Trough	Inoc	Thel	Lacc	Heb	Total
11/12	0	0	0	0	0
13/14	1	0	0	1	2
15/16	0	0	0	4	4
17/18	0	0	0	7	7
19/20	1	0	0	2	3
21/22	1	0	0	2	3
23/24	0	0	0	0	0
25/26	0	0	0	0	0
Total	3	0	0	16	19

1986 Trough	Inoc	Thel	Lacc	Heb	Total
11/12	0	0	0	0	0
13/14	0	0	0	0	0
15/16	0	0	0	0	0
17/18	2	0	0	0	2
19/20	0	0	0	3	3
21/22	2	0	0	2	4
23/24	2	0	0	0	2
25/26	0	0	0	0	0
Total	6	0	0	5	11

similar number of Inocybe-type mycorrhizas. Over the whole 3 -year period, all troughs supported fruiting of *Inocybe* at one time or another, and 5 supported *Hebeloma* fruitbodies. Only 3 of the 8 troughs supported fruiting by one fungus or another in all of the three growing seasons.

The times of fruiting during the 3-year period are shown in Table 4.3.13. Of particular interest is the fact that many fruitbodies appeared in December and January of 1983, when the troughs had been set up for only 3-4 months and plant growth had been manipulated artificially by control of heating and daylength in the glasshouse. Thereafter, fruiting occurred in response to natural seasonal factors, most often in August and September of each year, although a large proportion of the *Inocybe* fruitbodies occurred earlier, in June of 1984 and 1986. Fruitbodies never developed in July, so in two of the three years there was evidence of a bimodal distribution of fruiting.

Occurrence of mycorrhizas on young seedlings. Of the 28 seedlings inserted down the sides of the troughs in June 1986, 26 survived until they were sampled in October 1986. Some of these seedlings originally bore mycorrhizas of *Hebeloma*, some of *Thelephora*, and some were non-mycorrhizal when planted in the troughs. Also, they were placed purposefully in selected positions in the troughs, namely where there was a predominance of Hebeloma-type or Lactarius-type mycorrhizas (as determined visually). The subsequent mycorrhizal status of these seedlings is shown in Tables 4.3.14 - 4.3.16.

In October 1986, seedlings had, on average, 235 root tips, of which a mean of 54% were mycorrhizal. The mycorrhizal types occurring on the seedlings were of the same range as those occurring on the older trees - mainly Hebeloma-, Inocybe-, Thelephora- and Lactarius-types, with some Cenococcum-type and, in a few instances, Laccaria-, Leccinum- and

Table 4.3.13
Experiment 1 - Times of fruitbody appearance

1983/84

Oct	Nov	Dec	Jan	Feb	// June	July	Aug	Sept	Oct
24	20	13	5,11	27	6,20		10	14	23
1I	4I	16I	19I	2I	11I		2I	2I	3I
		3T	4T	4T				1T	
		2L	4L	2L					7H

1985

June	July	Aug	Sept	Oct
		23,28	20	1
		1I	1I	1I
		1H	8H	6H

1986

June	July	Aug	Sept	Oct
23		13,20	16	
2I		2I	2I	
		4H	1H	

I = *Inocybe* spp.
T = *Thelephora* spp.
L = *Laccaria* spp.
H = *Hebeloma* spp.

Table 4.3.14
Mycorrhizal development on seedlings inoculated with *Hebeloma crustuliniforme*

Tree/ position	Total myco	Heb	Inoc	Thel	Lact	Lacc	Cen	Lec	Pax
12/2	31.2	13.1	0	10.5	0	0	0	0	0
12/4	17.5	17.5	0	0	0	0	0	0	0
16/2	60.8	7.4	0	15.2	38.2	0	0	0	0
20/3	50.2	3.9	17.4	0	11.1	0	17.8	0	0
26/2	67.7	0	29.8	36.5	0	0	1.4	0	0
15/1	45.3	4.2	3.6	0	37.5	0	0	0	0
21/3	63.4	3.7	38.4	0	14.8	6.5	0	0	0
22/1	51.9	13.1	10.3	0	27.4	0	1.1	0	0
23/1	66.3	7.4	25.3	21.4	12.2	0	0	0	0

Table 4.3.15
Mycorrhizal development on seedlings inoculated with *Thelephora terrestris*

Tree/ position	Total myco	Heb	Inoc	Thel	Lact	Lacc	Cen	Lec	Pax
16/1	10.9	6.2	4.7	0	0	0	0	0	0
23/2	39.2	0	24.3	7.0	1.7	0	6.2	0	0
26/3	72.6	0	15.8	19.9	26.7	0	9.1	0	1.1
21/2	86.4	0	41.6	38.4	6.4	0	0	0	0
21/4	93.6	0	41.6	0	23.4	5.2	23.4	0	0
23/3	50.6	0	36.4	12.1	0	0	2.1	0	0
23/4	72.8	0	0	72.8	0	0	0	0	0

Table 4.3.16

Uninoculated seedlings

Tree/ position	Total myco	Heb	Inoc	Thel	Lact	Lacc	Cen	Lec	Pax
19/4	58.5	8.0	9.0	7.4	34.1	0	0	0	0
20/3	27.3	1.2	10.2	0	8.2	0	7.7	0	0
21/1	75.2	0	13.8	20.7	40.7	0	0	0	0
25/2	88.1	0	10.5	45.1	17.3	0	0	0	15.2
25/3	81.6	0	41.2	29.6	10.8	0	0	0	0
11/2	18.4	11.0	0	7.4	0	0	0	0	0
15/2	36.3	14.0	0	0	21.9	0	0	0	0
15/3	12.6	0	7.6	0	5.0	0	0	0	0
23/4	53.9	3.4	28.9	12.3	1.7	0	1.7	5.9	0
25/1	76.5	0	70.6	5.9	0	0	0	0	0

Paxillus-types. The table below shows the number of seedlings irrespective of treatment that had root tips attributable to each mycorrhizal type.

Type	No. of seedlings (max.26)	Mean % infection *
Hebeloma	14	4.4
Inocybe	20	18.5
Thelephora	16	13.9
Lactarius	18	13.0
Laccaria	2	0.5
Cenococcum	10	3.0
Leccinum	1	0.2
Paxillus	2	0.6

(* on seedlings that bore the representative mycorrhizal type)

Nine seedlings originally had at least 50% of their root tips mycorrhizal with *Hebeloma* when they were inserted in the troughs. By October 1986, however, they had only 7.8 ± 1.9% root tips mycorrhizal with *Hebeloma*. This was still significantly more (P=0.05) than for seedlings that had not previously been infected with *Hebeloma* (mean 2.6 ± 1.1%). Similarly, for the seven seedlings that originally had at least 50% of their root tips mycorrhizal with *Thelephora*, the percentage occurrence of this mycorrhizal type had declined to only 21.5 ± 9.9% by October 1986. This value was greater than that for seedlings not originally infected by *Thelephora* (mean 9.1 ± 2.8%) but the difference was not significant when analysed by Student's t-test. The ten seedlings that were non-mycorrhizal when they were inserted into the troughs became infected predominantly by *Inocybe* (mean 19.2 ± 6.9%), *Lactarius* (mean 14.0 ± 4.5%) and *Thelephora* (mean 12.8 ± 4.7%) as shown in Table 4.3.16.

The positions into which seedlings were planted, i.e. whether in regions with a predominance of Hebeloma- or Lactarius-type mycorrhizas, had no significant effect on the development of mycorrhizal types on the seedlings,

except in the case of Inocybe-type. Significantly (P = 0.01) more Inocybe-type developed on the roots of seedlings in positions with *Lactarius* visible on the surface (mean 23.4 ± -6.%1) than in positions with *Hebeloma* visible on the surface (mean 13.6 ± 3.4%); the older tree roots also reflected this difference (P=0.05) when the slices of soil, beside which the seedlings were inserted, were assessed microscopically (mean occurrence of Inocybe-type mycorrhizas 13 and 7% respectively). The occurrence of existing mycorrhizas of *Hebeloma* or *Thelephora* on the seedlings evidently did not influence the subsequent development of mycorrhizas on the seedling root systems, because mycorrhizas of *Inocybe*, *Thelephora* and *Lactarius* developed with more or less equal facility on the seedling roots irrespective of previously established mycorrhizas.

4.4. Experiment 2.

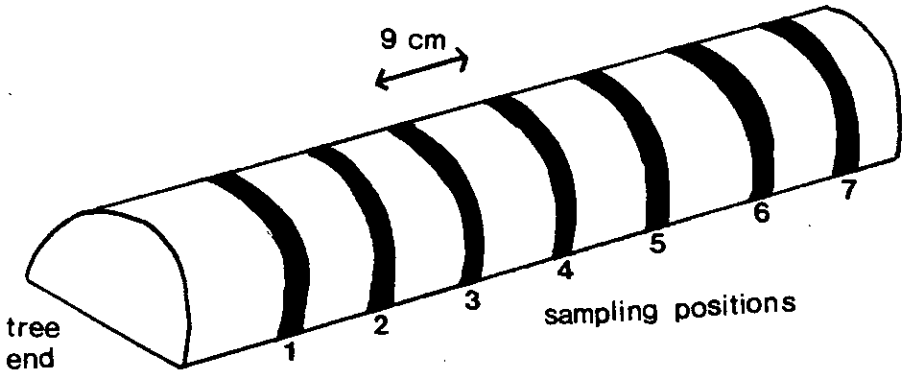
4.4.1. Method.

This experiment comprised 40 troughs set up in March 1984 as described in Section 4.2.1, and one birch sapling was planted into each trough. The troughs were divided into eight groups and each group was inoculated in seven positions along the lower surface with liquid-grown inoculum of *Laccaria proxima* isolate 19, *Hebeloma crustuliniforme* isolate 4 or *Lactarius pubescens* isolate 4 in the patterns shown in Fig 4.4.1 Thereafter, the inoculations were repeated in identical patterns in September 1984 and again in March 1985 (as illustrated in Fig 4.4.2) when root extension resumed after dormancy.

In March 1985 one aseptically germinated birch seedling was inserted into the soil at the positions of inoculation in each trough in order to test the infectivity of the applied inoculum towards seedling roots in the presence of the older tree roots. Seedlings that died within 4 weeks of planting were replaced with non-mycorrhizal seedlings of an equivalent age. Thereafter dead seedlings were not replaced. In June 1986 further non-mycorrhizal seedlings were inserted down the sides of the troughs (Section 4.2.5) at positions with dense development of mycorrhizas visible to the naked eye; this was in order to investigate the infectivity of established mycorrhizas on older roots towards seedling roots.

As with the troughs in Experiment 1, the troughs in this experiment were arranged in racks in a large unheated, but frost-free, glasshouse which was cooled in summer by thermostatically-controlled vents and a fan. The troughs remained in this glasshouse for the duration of the experiment and were watered as required.

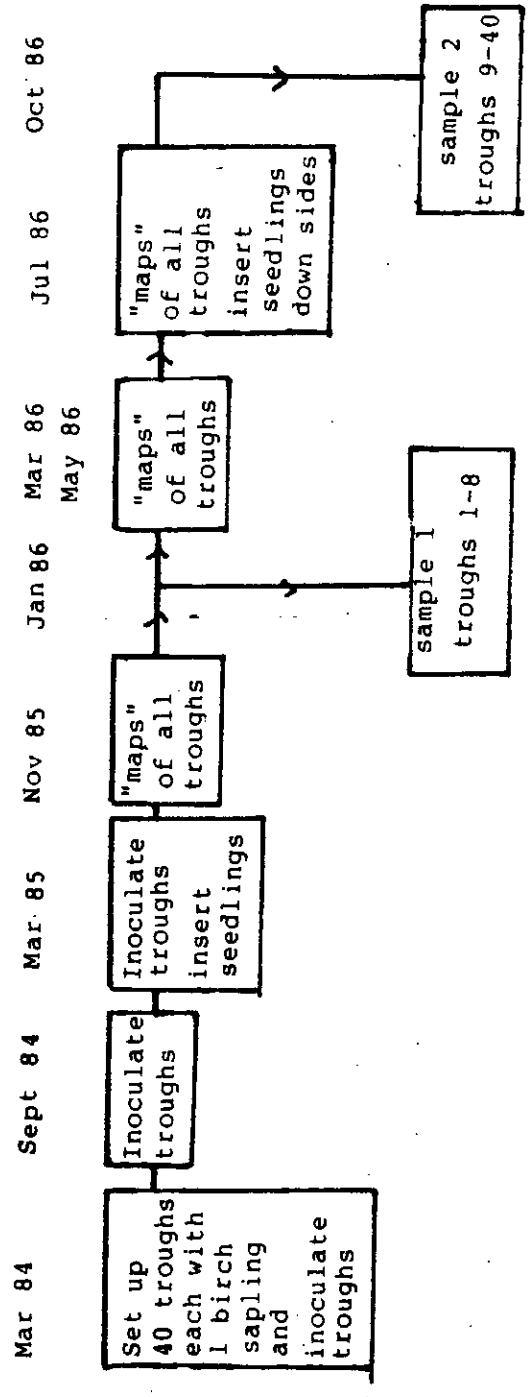
Fig 4.4.1
Positions and types of liquid-culture inocula applied to troughs in Experiment 2.



Troughs	Inoculation in sampling position						
	1	2	3	4	5	6	7
1, 9 - 12	Lpx	Hc	Lpub	Lpx	Hc	Lpub	Lpx
2,13 - 16	Hc	Lpub	Lpx	Hc	Lpub	Lpx	Hc
3,17 - 20	Lpub	Lpx	Hc	Lpub	Lpx	Hc	Lpub
4,21 - 24	Lpx	Lpx	Lpx	Lpx	Lpx	Lpx	Lpx
5,25 - 28	Hc	Hc	Hc	Hc	Hc	Hc	Hc
6,29 - 32	Lpub	Lpub	Lpub	Lpub	Lpub	Lpub	Lpub
7,33 - 36	mix	mix	mix	mix	mix	mix	mix (*)
8,37 - 40	-	-	-	-	-	-	-

(*, mix = one aliquot of Lpx, Hc and Lpub inocula
- = no inoculum added, sterile water only added
Lpx = *Laccaria proxima* isolate 19
Hc = *Hebeloma crustuliniforme* isolate 4
Lpub = *Lactarius pubescens* isolate 4

Fig 4.4.2
Flowchart showing experimental procedure for Experiment 2



The visible mycorrhizas on the lower surface of the troughs (Section 4.2.3) were recorded periodically and fruitbodies appearing on the troughs were identified and their positions recorded prior to removal (Section 4.2.4).

In January 1986 a representative sample of the troughs was destructively sampled; the remainder of the troughs was incubated until October 1986, when they were sampled in a similar manner (Fig 4.4.2). Surviving seedlings were removed and their roots also were assessed for mycorrhizal development.

Eight troughs, representing one from each pattern of inoculation, were sampled in January 1986 as described in Section 4.2.6. The total numbers of root tips were recorded in each of the seven sampling positions along the troughs (Table 4.4.1), as were the percentages of root tips bearing any type of mycorrhiza (Table 4.4.2) or mycorrhizas identified as belonging to *Hebeloma*, *Lactarius*, *Laccaria*, *Inocybe*, *Thelephora*, *Paxillus*, *Cenococcum* and *Leccinum* (Tables 4.4.3 - 4.4.9). The small seedlings that had survived in the troughs were also assessed for mycorrhizal development (Table 4.4.10). Immediately prior to these destructive samplings, the soil surface on the bottom of each trough was examined, and the distribution of mycorrhizal types visible to the naked eye was recorded (Fig 4.4.3).

4.4.2. Results - Sample 1.

Distribution of root tips. As shown in Table 4.4.1, the numbers of root tips recorded in the different troughs varied considerably. Nevertheless, analysis of variance of the data showed that, overall, there were significantly (P=0.05) more root tips in the two sampling positions closest to the tree bases than in the other sampling positions. The percentage of roots that became mycorrhizal did not, however, differ significantly along the lengths of the troughs, although the percentage tended to be higher in sampling positions further from, as

Tables 4.4.1 - 4.4.9
Experiment 2. Growth and mycorrhizal development of roots of birch saplings grown in troughs (see text for details) and sampled in January 1986.

Table 4.4.1
Number of root tips per sampling position

Trough	Sampling position						
	1	2	3	4	5	6	7
1	474	1400	614	536	665	221	414
2	1231	2206	1406	549	1244	989	815
3	1374	678	592	525	1041	758	1107
4	1577	1728	1285	559	740	436	771
5	2807	1907	917	1096	1207	875	704
6	1050	1043	833	119	170	235	189
7	714	383	332	394	398	145	302
8	641	1026	1070	514	203	303	106
Mean	1231	1297	881	536	707	395	551

SED 236
5%LSD 477

Table 4.4.2
Percentage mycorrhizal root tips per sampling position

Trough	Sampling position						
	1	2	3	4	5	6	7
1	34.7	58.2	49.7	68.5	87.5	89.1	92.7
2	44.1	60.3	41.7	42.8	57.9	62.0	61.9
3	70.4	54.7	62.4	42.0	67.1	64.8	56.4
4	76.5	68.5	54.8	82.0	79.1	92.9	78.1
5	58.3	45.8	58.4	55.5	45.1	26.4	32.2
6	53.3	50.5	64.7	77.3	69.9	79.6	61.3
7	45.8	68.0	64.5	67.4	92.4	91.3	99.9
8	100.0	99.9	100.0	60.9	99.9	100.0	99.9
Mean	60.4	63.2	62.0	62.2	74.9	75.8	72.8

SED 9.9
5%LSD -

opposed to nearer to, the tree bases (Table 4.4.2).

Development of inoculant-type mycorrhizas. The relationship between positions of inoculation of the troughs and the positions in which mycorrhizas of the inoculant types (Hebeloma, Laccaria and Lactarius) developed is shown in Tables 4.4.3 - 4.4.5 Mycorrhizas of both *Hebeloma* and *Lactarius* occurred in significantly more sampling positions to which inoculum of these fungi had been applied. Indeed, both of these mycorrhizal types were absent from the three troughs that had not recieved the appropriate inoculum at some point along their length, i.e. troughs 4, 6 and 8 for *Hebeloma* and 4, 5 and 8 for *Lactarius* (Tables 4.4.3 and 4.4.4). In contrast, the occurrence of Laccaria type mycorrhizas (Table 4.4.5) showed no obvious relationship to positions in which *Laccaria* inoculum had been applied. Rather, this mycorrhizal type was found along most of the length of two troughs, one of which had been inoculated with *Laccaria* (trough 4) and one of which had not been so (trough 6). It occurred in only two positions in a third trough (7), which had been inoculated along its length with *Laccaria*, *Hebeloma* and *Lactarius* inocula. So, there was evidence that Hebeloma- and Lactarius-type mycorrhizas had developed at least partly in response to inoculation with these fungi, whereas Laccaria-type mycorrhizas apparently developed at random.

Development of other mycorrhizal types. Several mycorrhizal types developed from either glasshouse-borne inoculum or that initially present on the plants from their field site. Of these other types, Thelephora-type was most common (23.7 % of all root tips), followed by Inocybe-type (18.8 % of tips), although these mycorrhizal types occurred in similar numbers of sampling positions overall (49 and 53 of the total 56 positions respectively). Both of these mycorrhizal types were generally distributed along the lengths of the troughs,

Table 4.4.3
Percentage of root tips attributable to Hebeloma

Trough	Sampling position						
	1	2	3	4	5	6	7
1	2.9	12.6	7.0	0	0	0	0
2	0	0	0	14.2	39.2	41.2	28.5
3	6.4	2.8	17.9	3.2	0	4.0	7.9
4	0	0	0	0	0	0	0
5	47.0	39.4	25.8	2.8	0	0	0
6	0	0	0	0	0	0	0
7	0.1	3.1	0.1	12.2	0	2.7	0
8	0	0	0	0	0	0	0

Table 4.4.4
Percentage root tips attributable to Lactarius

Trough	Sampling position						
	1	2	3	4	5	6	7
1	0	0	4.1	5.4	0	0	0
2	0	2.4	0	0	0	0	0
3	8.8	0	0	7.8	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	1.8	0.5	0.3	0	0	0	0
7	0	0.1	4.8	0	0	0	0
8	0	0	0	0	0	0	0

= sampling positions inoculated with *Hebeloma*
= " " " " *Lactarius*

Table 4.4.5
Percentage root tips attributable to Laccaria

Trough	Sampling position						
	1	2	3	4	5	6	7
1	**0**	0	0	**0**	0	0	**0**
2	0	0	**0**	0	0	**0**	0
3	0	**0**	0	0	**0**	0	0
4	**8.9**	**7.1**	**1.8**	**6.1**	**0**	**1.0**	**3.4**
5	0	0	0	0	0	0	0
6	3.2	5.6	8.9	0	8.2	12.8	0
7	**0.1**	**0**	**0**	**0**	**0**	**0**	**1.6**
8	0	0	0	0	0	0	0

□ = positions inoculated with *Laccaria*

with no significant difference in occurrence between sampling positions for *Thelephora* and only relatively minor variations in distribution in the case of Inocybe-type (which was most common in sampling position 3). Mycorrhizas of *Paxillus*, *Cenococcum* and *Leccinum* (Tables 4.4.8 and 4.4.9) were relatively uncommon although in four troughs (1,2,7 and 8) Paxillus-type mycorrhizas represented a substantial proportion of the mycorrhizal tips in one or more of the sampling positions (Table 4.4.8); indeed the root systems of three plants (troughs 1,7 and 8) had an abundance of Paxillus-type mycorrhizas in the youngest regions, that is in sampling position 7. In view of the variation in occurrence of mycorrhizas of *Paxillus*, *Cenococcum* and *Leccinum*, both between troughs and between sampling positions, it is likely that these fungi developed from inoculum present on the root systems when the plants were collected from the field site.

Observed distributions of mycorrhizal types on the lower soil surface

Only mycorrhizas of *Hebeloma* and *Lactarius* were sufficiently distinctive to be identifiable by visual inspection of the soil on the bottoms of the troughs, and the percentage surface cover by these types was relatively low (Fig 4.4.3).Hebeloma-type mycorrhizas were observed in 15 of the total 56 "sampling" positions corresponding to those in which microscopic assessments were subsequently made. In 12 of these 15 "positive" positions Hebeloma-type mycorrhizas were subsequently recorded microscopically in soil samples (at a mean frequency of 18.6 $\pm$ 4.5% of all root tips); the other 3 positions did not reveal Hebeloma-type mycorrhizas by microscopy. Visual assessment failed to detect Hebeloma-type mycorrhizas in 10 other sampling positions where these mycorrhizas were subsequently recorded microscopically, but the mean frequency of occurrence assessed by microscopy was only 9.7 + 4.4% in these cases. So the visual assessment on the lower soil surface detected the major positions of

Table 4.4.6
Percentage of root tips attributable to Inocybe

Trough	Sampling position						
	1	2	3	4	5	6	7
1	4.0	11.2	32.6	22.0	11.1	0	0
2	19.2	23.0	13.1	15.7	9.6	13.0	10.8
3	0.1	10.3	13.3	16.9	17.4	45.1	28.6
4	26.7	20.8	26.0	33.4	39.3	24.5	31.0
5	1.7	0.7	23.2	15.2	10.3	6.3	4.1
6	13.2	23.6	23.2	2.5	30.6	36.2	25.9
7	1.1	35.8	37.6	5.8	4.9	15.9	0
8	16.4	53.4	51.5	32.3	39.9	11.2	11.3

Table 4.4.7
Percentage root tips attributable to Thelephora

Trough	Sampling position						
	1	2	3	4	5	6	7
1	27.8	32.3	6.0	26.7	1.5	0	0
2	28.5	10.8	22.6	0	0	0	0
3	1.1	6.8	31.2	14.1	49.7	15.6	15.5
4	27.2	25.1	24.9	42.7	31.8	67.4	43.7
5	9.6	5.7	9.4	38.5	34.8	20.1	28.1
6	8.7	20.5	32.3	74.8	31.1	30.6	26.4
7	34.4	28.9	22.0	39.1	29.6	1.0	0
8	12.2	41.0	32.5	15.8	34.9	46.9	66.0

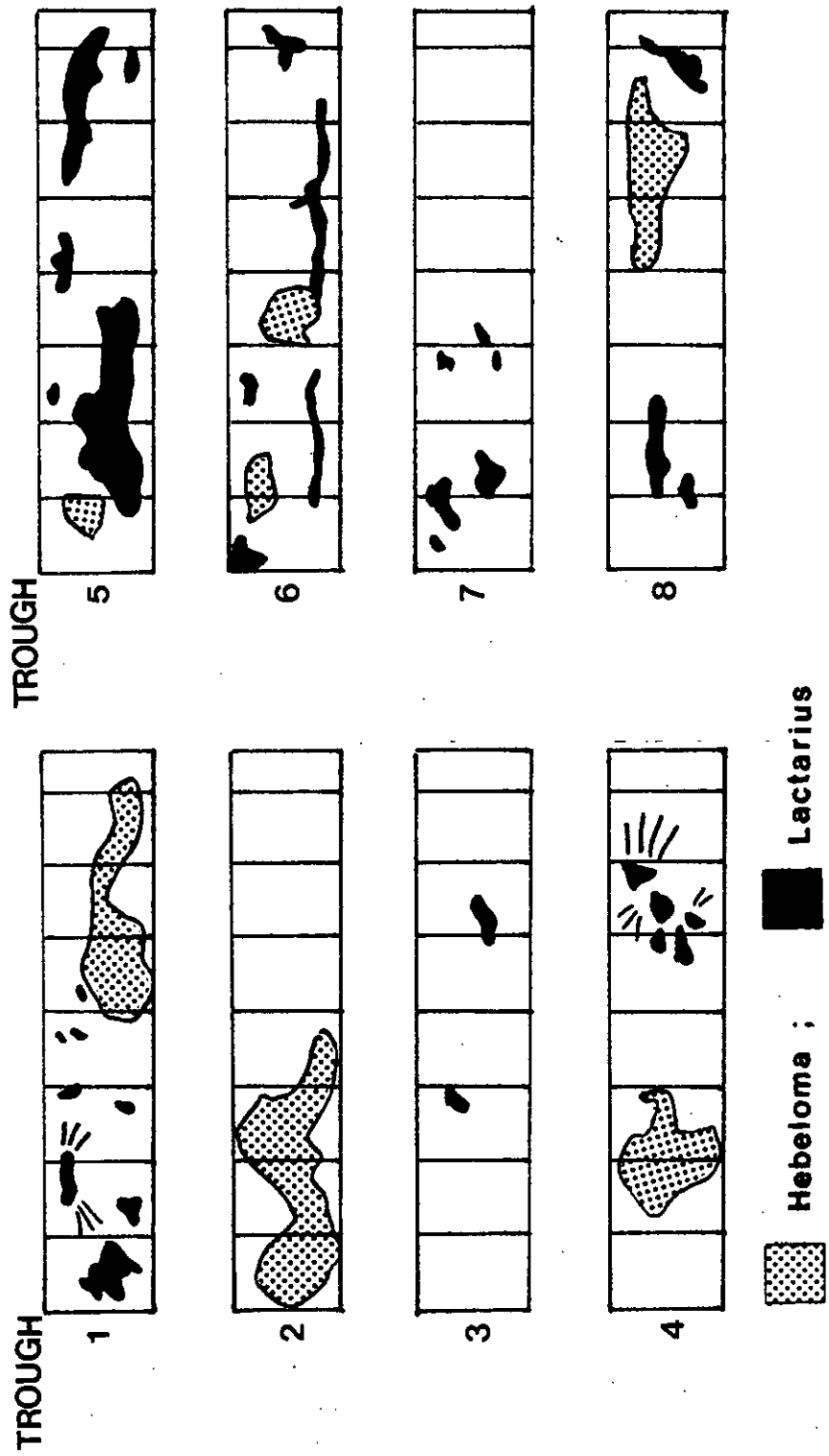
Table 4.4.8
Percentage root tips attributable to Paxillus

Trough	Sampling position						
	1	2	3	4	5	6	7
1	0	0.5	0	14.4	74.9	89.1	92.7
2	10.8	23.3	12.2	0	0	0	0
3	4.3	0	0	0	0	0	4.4
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	0	0	0	0	0	0	9.0
7	0	0.1	0	6.8	51.7	71.7	98.3
8	8.1	5.5	8.4	12.8	25.1	41.9	22.6

Table 4.4.9
Percentage root tips attributable to Cenococcum/Leccinum

Trough	Sampling position						
	1	2	3	4	5	6	7
1	0/0	1.6 /0	0/0	0/0	0/0	0/0	0/0
2	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3	0 /49.7	0 /34.8	0/0	0/0	0/0	0/0	0/0
4	13.7 /0	15.5 /0	2.1 /0	0/0	0/0	0/0	0/0
5	0/0	0/0	0/0	0/0	0/0	0/0	0/0
6	0	0	0/0	0/0	0/0	0/0	0/0
7	0.1 /0	0/0	0/0	3.5 /0	0 /6.2	0/0	0/0
8	6.3 /0	0/0	7.6 /0	0/0	0/0	0/0	0/0

Fig 4.4.3
"Maps" of distribution of mycorrhizal types visible on the lower surface to troughs 1 - 8, each containing one birch sapling and sampled in January 1986.



mycorrhizal development by *Hebeloma*

Lactarius -type mycorrhizas were observed on the lower soil surface in 24 sampling positions but in only 6 of these were Lactarius-type mycorrhizas subsequently seen by microscopy. Visual inspection failed to detect the occurrence of Lactarius-type mycorrhizas in 4 sampling positions where they were subsequently seen by microscopy. There was, therefore, very poor agreement between the results of visual and microscopical assessments of Lactarius-type mycorrhizas, perhaps owing to difficulties of identification of these mycorrhizas by visual means as discussed more fully in Section 4.4.3 However, Lactarius-type mycorrhizas were present in only low numbers in the troughs at this stage of the experiment (Table 4.4.4), and visual inspection would not be expected easily to detect such low numbers. A notable feature seen in Fig 4.4.3 is that obvious development of Hebeloma-type mycorrhizas and what were considered to be Lactarius-type mycorrhizas seldom coincided. In other words, the "maps" show evidence of contrasting distribution of the visually assessed mycorrhizal types.

Mycorrhizal development on seedlings. Only 18 of the 56 non-mycorrhizal seedlings planted originally survived until November 1985. Of these seedlings most developed mycorrhizas predominantly of *Inocybe* and *Thelephora* when assessed microscopically, and only occasionally of *Hebeloma*, *Laccaria*, *Cenococcum* and *Paxillus* (Table 4.4.10). Unfortunately, the poor survival of the seedlings precludes detailed comparisons of their mycorrhizal status with that of the older tree root systems. However it is notable that in instances in which Hebeloma-type mycorrhizas developed well on the seedlings there was correspondingly good development of Hebeloma-type mycorrhizas on the larger tree root systems (Table 4.4.3).

Table 4.4.10
Summary of mycorrhizal development on the roots of seedlings inserted into troughs containing birch saplings and sampled in January 1986 (% infection).

Tree/ position	Heb	Inoc	Thel	Lacc	Cen	Pax
1/1	8.7	3.6	2.5	0	0	0
1/4	4.1	15.1	0	0	0	0
2/1	0	0	0	0	0	0
2/4	22.1	0	32.3	0	0	0
3/2	0	22.1	41.9	0	0	0
3/3	0	0	54.4	0	0	0
3/4	0	40.0	14.2	0	0	0
4/2	0	32.5	9.3	0	30.2	0
4/3	0	17.4	24.2	0	0	0
4/5	0	63.6	0	18.2	0	0
5/3	38.9	5.5	3.7	0	0	0
5/6	0	2.6	40.3	0	0	0
5/7	0	0	39.5	0	0	0
7/1	0	2.6	55.8	6.5	0	0
7/4	25.0	4.2	31.2	0	0	0
8/1	0	28.1	38.6	0	0	0
8/2	0	50.8	42.9	0	0	0
8/3	0	44.2	10.8	2.3	0	24.0

[ ] = positions inoculated with Hebeloma
[ ] = positions inoculated with Laccaria

4.4.3 Results - Sample 2.

The remaining 32 troughs in the experiment were sampled in October 1986. Figure 4.4.4 shows the distribution of root tips along the troughs in the experiment as a whole. As might have been expected, the largest numbers of tips occurred in the sampling positions closest to the tree bases, but the numbers then fell to a more or less constant level with increasing distance from the tree bases. A mean of at least 600 tips was counted in soil sections from each of the seven sampling positions of each trough, and on this basis it was decided that distributions of different mycorrhizal types along the troughs could accurately be compared as percentages of all root tips infected by the different fungi. Although the number of root tips differed substantially between troughs, ranging from a total of 1317 for the seven sampling positions in trough 36 to 10,909 for the seven positions in trough 31, yet there was no relationship between this and the applied treatments.

The percentage of root tips that was infected by any type of mycorrhizal fungus in each trough is shown in Table 4.4.11. The percentage differed substantially between troughs but was not related to treatment differences. The percentage did not differ between sampling positions in the experiment as a whole, being more or less 60 percent in all positions.

Tables 4.4.12 - 4.4.18 show the percentages of root tips that were infected with specific identifiable mycorrhizal types in each of the seven sampling positions along each trough at the final sampling. Tables 4.4.12 - 4.4.14 also show the positions at which inocula of *Laccaria proxima*, *Hebeloma crustuliniforme* and *Lactarius pubescens* were applied to the troughs. The tables enable comparisons to be made readily between treatments and also enable

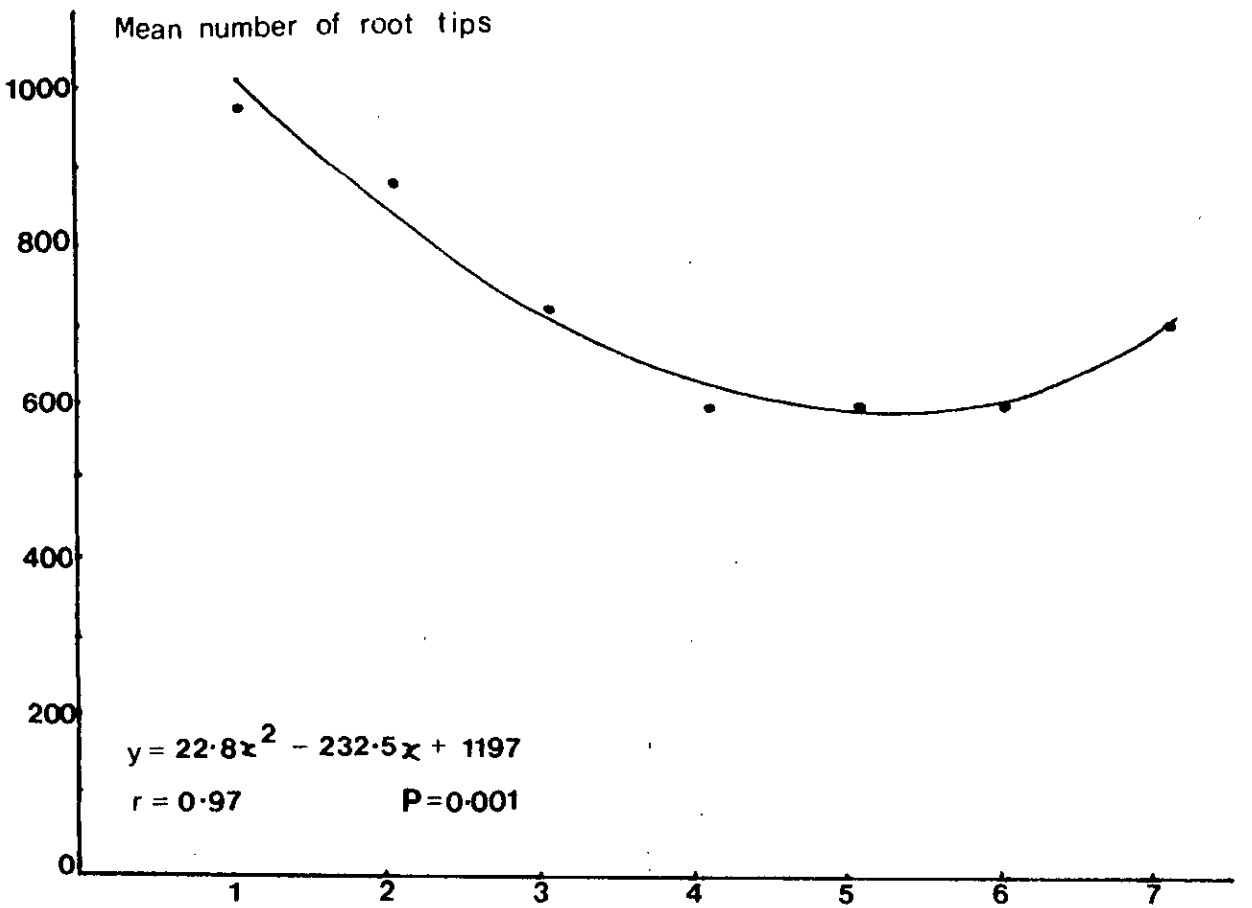


Fig 4.4.4
Distribution of root tips in October 1986 in each sampling position (means of 32 positions) with equation of line, correlation coefficient (r) and level of significance.

patterns of mycorrhizal development to be compared in different positions in the troughs, irrespective of treatment. In the following account a number of comparisons are made and are tested statistically, based on appropriate parts of the data in Tables 4.4.12 - 4.4.14.

Development of mycorrhizas of inoculant types. The development of inoculant-type mycorrhizas, namely those attributable to *Laccaria*, *Hebeloma* and *Lactarius,* is shown in Tables 4.4.12 - 4.4.14. A number of general points are apparent in these tables. Firstly, in the experiment as a whole, Hebeloma-type mycorrhizas were more commonly encountered (in 173 of 224 sampling positions) than were mycorrhizas of *Lactarius* (in 76 of 224 sampling positions) and these in turn were more common than were Laccaria-type mycorrhizas (in 47 of 224 sampling positions). However, not all of the Hebeloma-type mycorrhizas developed from the added inoculum, because some were present in troughs that had not received *Hebeloma* inoculum, as explained later. The same was true, to a lesser degree, for Laccaria and Lactarius-type mycorrhizas. Considering only those sampling positions in which each inoculant mycorrhizal type occurred, Hebeloma-type represented a mean 16 percent of all root tips in these positions, compared with 7 percent for Lactarius-type and 1 percent for Laccaria-type. So, again, *Hebeloma* was the dominant inoculant-type mycorrhiza, followed by *Lactarius* and then *Laccaria*-type.

A third general point seen in Tables 4.4.12 - 4.4.14 is that all three inoculant-type mycorrhizas occurred together in only 4 (i.e.1%) of the total 224 sampling positions in the 32 troughs as a whole. Also, a low proportion of sampling positions (20 of the total 224, or 8.9%) did not contain mycorrhizas of any of these types. Most positions (117 of 224, or 52.2%) contained only one of the inoculant-type mycorrhizas, but many (83 of 224, or 37.1%) contained two of

Tables 4.4.11 - 4.4.18
Experiment 2. Mycorrhizal development on roots of birch saplings grown in troughs (see text for details) and sampled in October 1986.

Table 4.4.11
Percentage mycorrhizal root tips

Trough	Sampling position						
	1	2	3	4	5	6	7
9	86.8	84.6	64.6	73.9	84.1	82.7	79.5
10	37.6	42.5	62.3	62.1	65.3	85.0	62.6
11	72.1	88.4	90.2	91.1	92.5	86.3	86.1
12	32.8	39.8	30.6	35.2	31.2	39.7	54.6
13	64.9	63.7	45.7	43.1	60.6	70.7	51.5
14	79.6	75.1	52.5	46.5	44.1	57.9	30.0
15	77.1	70.9	53.5	53.5	81.7	88.3	83.1
16	56.5	45.5	58.6	41.5	51.8	77.4	51.6
17	56.1	38.5	52.8	67.8	68.2	53.9	72.5
18	83.9	74.3	61.2	62.5	64.2	66.1	76.4
19	50.8	52.2	81.3	58.5	68.5	58.9	36.1
20	15.7	58.2	25.1	29.7	27.9	29.1	45.9
21	42.1	51.5	74.1	85.8	41.9	47.7	55.8
22	71.6	77.8	36.5	22.5	55.9	47.1	45.6
23	66.2	59.9	69.7	59.7	36.5	37.7	39.1
24	33.2	57.1	55.2	50.2	69.1	52.2	60.2
25	74.4	80.3	96.1	76.2	81.3	50.4	79.5
26	57.6	55.5	54.1	64.5	71.5	53.3	63.7
27	52.7	62.1	80.9	70.4	66.9	48.6	66.9
28	54.1	45.5	57.0	54.5	67.9	56.1	72.5
29	20.7	40.4	35.5	30.5	33.4	26.3	52.3
30	76.6	82.0	66.2	82.6	64.1	39.6	60.1
31	84.2	49.4	51.6	37.3	77.2	73.1	87.6
32	72.4	84.4	70.8	56.9	48.4	41.4	56.1
33	60.3	53.9	61.7	61.6	48.2	40.9	35.4
34	90.3	82.6	87.8	94.0	83.5	86.7	84.5
35	54.4	69.0	78.3	90.3	73.6	84.1	79.7
36	62.7	51.7	39.2	32.9	19.1	15.4	20.4
37	53.9	57.1	43.8	54.8	47.5	60.2	69.3
38	55.5	65.0	90.1	90.4	78.1	68.4	92.5
39	63.4	60.8	77.8	86.6	79.8	90.7	75.9
40	49.4	41.2	59.2	56.3	66.8	57.1	71.1
Mean	59.7	60.9	61.4	60.1	60.9	58.5	62.4

Table 4.4.12
Percentage root tips attributable to Laccaria

Tree	Sampling position						
	1	2	3	4	5	6	7
9	0	0	0	7.9	1.2	0	9.3
10	0	0	6.3	0	4.2	1.8	22.6
11	0	0	0	7.9	1.2	0	9.3
12	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0
15	0	0	0	0	4.7	2.7	3.3
16	0	0	0	0	0	0.4	0
17	0	0.7	0	0	0	4.5	0
18	3.5	0	8.2	0	0	0	0
19	0	0	0	0	2.2	0	0
20	0	0	0	0	0	0	0
21	0	0	0	0	0	0	5.9
22	0	0	0	0	0	0	0
23	8.2	0.6	3.5	1.4	0	3.2	1.5
24	0	0	0	0.8	10.2	5.3	4.2
25	0	0	0	0	0	0	0
26	0	0	0	0	0	0	3.8
27	0	5.9	0	0	0	0	0
28	0	4.1	0	1.2	0	0	1.4
29	0	0	0	0	0	0	0
30	0	0	0	0	0	0.8	2.0
31	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0.5
33	2.3	0	1.7	1.3	0	0	0
34	0	0	0	0.9	0	0	4.6
35	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0
37	0	4.3	3.9	13.5	4.5	0	33.7
38	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0.3
40	0	0	0	0	0	0	0

= sampling positions inoculated with *Laccaria*

Table 4.4.13
Percentage root tips attributable to Hebeloma

Tree	Sampling position						
	1	2	3	4	5	6	7
9	0.2	0	5.9	11.2	5.0	35.0	35.8
10	14.4	12.6	15.6	0	0	29.4	13.0
11	7.7	3.3	0	0	26.1	13.0	19.6
12	0	9.5	1.0	0	9.1	10.0	14.2
13	36.2	26.8	0	7.4	0	21.2	0
14	55.9	58.6	30.3	27.5	20.8	37.3	4.9
15	13.7	21.5	16.7	17.6	10.6	38.0	34.4
16	0	0.9	0	10.1	5.1	26.3	22.6
17	11.6	0	3.2	6.9	21.0	7.8	0
18	37.7	10.9	6.3	0	21 7	15.9	16.0
19	5.6	20.8	19.3	19.3	32.5	7.5	13.7
20	5.8	12.9	12.1	4.3	3.7	4.7	8.5
21	17.4	5.3	6.8	2.1	0	0	6.9
22	18.2	2.0	4.3	0	0	0	0
23	8.6	36.3	46.1	17.9	8.1	8.6	8.7
24	0	37.1	19.3	6.3	16.5	0	0
25	0	18.2	13.1	8.9	6.7	26.3	34.5
26	43.2	33.8	21.7	13.8	39.2	7.9	1.8
27	8.0	11.2	6.0	8.7	22.1	8.9	24.9
28	0	0	0	5.7	16.9	18.1	8.7
29	0.8	6.5	9.8	9.6	15.6	8.9	20.4
30	0	0	0	11.7	0.9	6.6	12.8
31	0	7.7	0	0	0	0	0
32	5.8	14.0	14.8	7.0	1.4	1.1	0
33	0	4.1	0	0	9.7	8.8	6.3
34	7.2	17.1	18.1	38.1	0	0	0
35	38.4	31.7	26.2	31.8	70.4	81.4	64.2
36	37.8	17.9	14.7	3.8	3.6	6.9	3.1
37	1.6	0.6	0	0	0	10.9	0.6
38	31.9	36.9	26.4	25.5	25.4	30.6	32.5
39	0	0	3.7	7.4	12.9	12.1	12.5
40	0	0	0.4	5.0	21.2	21.5	21.8

[ ] = sampling positions inoculated with *Hebeloma*

Table 4.4.14
Percentage root tips attributable to Lactarius

Tree	Sampling position						
	1	2	3	4	5	6	7
9	0	0	0	0	0	2.4	0
10	0	0.9	0	0	0	0	0
11	8.3	15.5	20.3	7.9	1.9	1.6	0
12	0	0	0	0	3.6	0	0
13	0	0	0	13.6	18.4	0	0
14	0	0	0	0	0	0	0
15	2.0	0	7.7	3.3	0	0	0
16	34.1	12.8	36.8	18.8	37.3	2.2	4.0
17	0	0	0.8	0	0	0	0.8
18	0	18.9	18.2	11.4	0	3.7	5.0
19	17.0	0	0	0	0	0	0
20	4.8	29.2	2.0	9.7	12.8	0	17.0
21	0	0	0	0	0.8	4.0	0
22	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0
27	0	2.0	0	0	0	0	0
28	3.6	0	0	0	0	0	0
29	15.7	23.7	10.6	6.0	5.9	8.6	8.4
30	20.7	25.8	15.1	1.9	12.1	0	0
31	60.9	33.1	12.6	5.1	4.7	0	0
32	13.5	23.0	24.5	2.8	3.5	10.6	0.3
33	0	0	3.2	6.2	0.7	0	4.4
34	5.9	0.8	4.5	0	0	0.9	1.9
35	4.6	14.5	3.1	53.0	0	0	0
36	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0

[ ] = sampling positions inoculated with *Lactarius*

them. From the number of positions in which each mycorrhizal type occurred, as noted earlier, it is possible to calculate the expected frequencies at which 0,1,2, or 3 of these types would have occurred together by chance, and to compare these expected frequencies with those observed, as in the table below.

Number of sampling positions containing 0, 1, 2 or 3 inoculant-type mycorrhizas.

	0	1	2	3	Total
Observed	20.0	117.0	83.0	4.0	224
Expected	24.8	106.2	79.1	13.9	224

Analysis of this frequency distribution gives a chi-squared value of 9.27, significantly different from expectation (χ^2, 7.82) at P=0.05. Almost all of the deviation, however, is accounted for by the low incidence of sampling positions with all three mycorrhizal types; indeed the observed data (4) in this category alone are significantly different from expectation (13.9) at P=0.05.

Effect of inoculation. Even casual inspection of Tables 4.4.12 - 4.4.14 reveals that inoculant-type mycorrhizas (i.e those referable to *Laccaria, Hebeloma* and *Lactarius*) did not occur exclusively in troughs, or positions within troughs, in which inoculum of these fungi was added. Relevant data extracted from Tables 4.4.12-4.4.14 are shown below.

Number of sampling positions containing each mycorrhizal type.

Mycorrhizal types	Inoculated (total 84)	Uninoculated (total 140)	Significance of difference (χ^2)
Laccaria	24	23	P = nil
Hebeloma	70	103	P = nil
Lactarius	50	26	P = 0.001

Considering, first, the presence or absence of each mycorrhizal type in the sampling positions, and applying a null hypothesis that there is an equal

distribution between inoculated and uninoculated positions, χ^2 analysis reveals no evidence that the occurrence of Laccaria - or Hebeloma-type mycorrhizas was related to presence of absence of inoculation, but there was a highly significant effect of inoculation on the occurrence of Lactarius-type mycorrhizas.

The effects of inoculation were examined further by calculating the mean percentage of root tips attributable to each inoculant-type mycorrhiza in sampling positions to which inoculum had or had not been added. The following two tables show such percentages firstly including all zero values, and secondly, excluding zero values for the sampling positions. Comparisons between the means for inoculated and uninoculated positions have been analysed by Student's t-test.

Mean percentage of root tips with inoculant-type mycorrhizas in positions inoculated or not inoculated with the appropriate fungus.

Mycorrhizal type	Inoculated (total 84 positions)	Uninoculated (total 140 positions)	Significance of difference
Laccaria	1.22	0.84	P = nil
Hebeloma	15.74	10.99	P = 0.05
Lactarius	7.27	1.83	P = 0.001

When zero-values are included , as in the table above, it is again seen that the development of Laccaria-type mycorrhizas was independent of inoculation with *Laccaria proxima* and the development of Lactarius-type mycorrhizas was again significantly enhanced by inoculation with *Lactarius pubescens*. However it is also apparent that the development of Hebeloma-type mycorrhizas was enhanced by inoculation with *Hebeloma crustuliniforme* - an effect not revealed by the less sensitive χ^2 analysis applied earlier to the enumeration data. The following table (in which zero values have been excluded from the calculation) shows a somewhat surprising result, namely that there was no significant

increase in the degree of mycorrhizal development by any fungus in the presence of its inoculum compared with in the absence of inoculum. However, this anomaly can be explained by considering the spread of infection from inoculated positions in the troughs, as described below.

Mean numbers ± s.e. of mycorrhizas of Laccaria, Hebeloma and Lactarius in sampling positions to which inoculum of the appropriate fungus was applied (excluding zero values).

Fungus	Inoculated	Uninoculated	Significance of difference
Laccaria	4.3 ±1.0 (24 positions)	5.1 ±1.4 (23 positions)	P = nil
Hebeloma	18.9 ±1.9 (70 positions)	15.2 ±1.2 (103 positions)	P = nil
Lactarius	12.2 ±1.8 (50 positions)	9.9 ± 2.1 (26 positions)	P = nil

Spread of infection. No attempt was made to analyse spread of infection from applied inoculum of *L.proxima* because the analyses above showed no evidence that the inoculum was effective in establishing mycorrhizas. In the case of *Hebeloma* and *Lactarius,* however, the spread of infection from the inoculum could be analysed by comparing the mean percentage of mycorrhizas formed by these fungi in (1) uninoculated positions (uncoloured in Tables 4.4.12 - 4.4.14) in the first twelve troughs in Tables 4.4.12 - 4.4.14 to which inoculum of *Laccaria, Hebeloma* and *Lactarius* was applied in a repeating sequence and (2) all uncoloured positions in the remaining 20 troughs, to which no inoculum of the appropriate fungus had been added. The data, detailed below, show that for both *Hebeloma* and *Lactarius* there were significantly more mycorrhizas of these types in the uninoculated positions of the first twelve troughs (in which inoculum of the appropriate fungus was added in other positions) than in troughs to which

there was no added inoculum. This spread of mycorrhizal development from the positions of inoculation partly obscured the effects of inoculation on mycorrhizal abundance in the previous analysis.

Mean percentage of roots bearing mycorrhizas of *Hebeloma* or *Lactarius* in uninoculated positions in troughs to which inoculum of *Hebeloma* or *Lactarius* had or had not been applied elsewhere in the troughs.

Fungus	Inoculated troughs (56 positions)	Uninoculated troughs (84 positions)	Significance of difference (Student t-test)
Hebeloma	14.2	9.3	P = 0.05
Lactarius	4.4	0.1	P = 0.001

Distribution of mycorrhizas along the lengths of the troughs The distribution of mycorrhizas of *Laccaria, Hebeloma* or *Lactarius* (assessed as the number of positions , maximum 32, in which each type occurred) did not differ significantly between the seven sampling positions along the troughs when analysed by χ^2 test as shown below.

Observed occurrences (max.32) in different sampling positions.

Fungus	1	2	3	4	5	6	7	Calc χ^2 *	Signif. of diff.!
Laccaria	3	5	5	7	6	6	14	10.9	n.s.d
Hebeloma	22	26	24	24	25	27	28	1.1	n.s.d
Lact.	12	12	13	12	11	7	8	3.2	n.s.d

(! significance of difference ; * based on expectation of random distribution
Lact. = Lactarius)

However, when the mean percentages of mycorrhizas attributable to these fungi were calculated for the 32 replicate troughs at each sampling position, and subjected to analysis of variance, detailed below, then both *Laccaria* and *Lactarius* showed significant variation in their distribution between sampling

positions, whereas *Hebeloma* did not do so.

Mean percentage of mycorrhizas attributable to Laccaria, Hebeloma or Lactarius at different positions along the troughs.

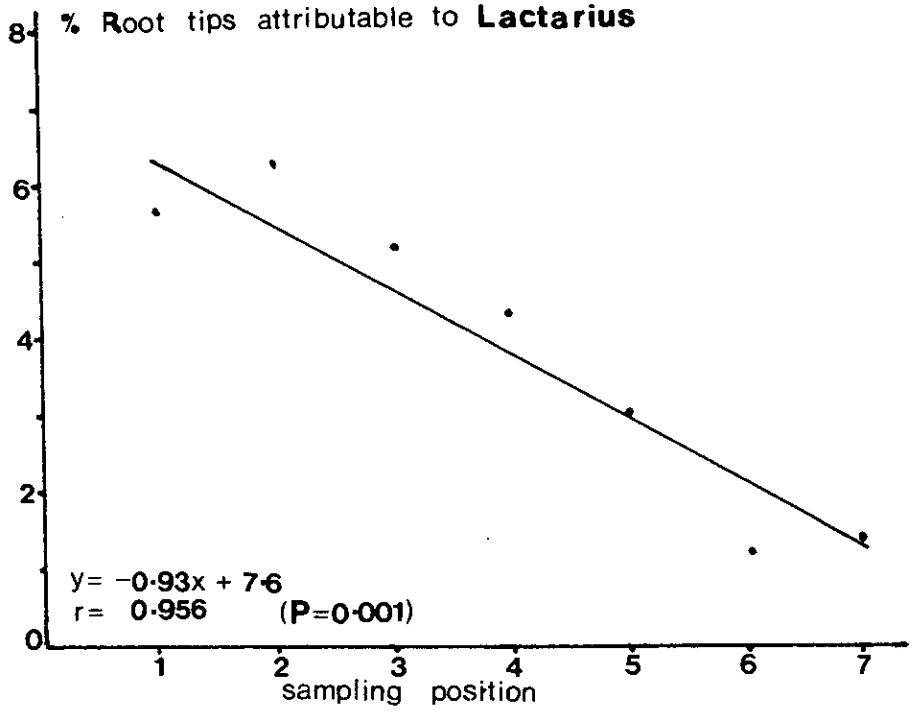
Fungus	1	2	3	4	5	6	7	SED	5% LSD	1% LSD
Lacc	0.4	0.5	0.7	0.8	0.8	0.6	2.9	0.8	1.6	2.3
Heb	12.7	14.3	10.7	9.6	13.3	15.8	13.8	3.5	-	-
Lact	5.9	6.3	4.9	4.4	5.2	1.1	1.3	2.2	4.5	-

(Lacc = Laccaria ; Heb = Hebeloma ; Lact = Lactarius
SED = standard error of the difference between any two means
LSD = least significant difference between any two means.)

The significant difference for *Laccaria* resulted mainly from the relatively large degree of development in sampling position 7, furthest from the tree base, which was significantly greater (P=0.05) than that in any of the other six positions. The significant difference for *Lactarius* was apparent only for positions 1 or 2 compared with positons 6 or 7. Nevertheless, the data above revealed a clear trend towards a reduction in the mean percentage of mycorrhizas of *Lactarius* with increasing distance from the tree base. This trend is clearly seen in Fig 4.4.5, there being a very highly significant linear relationship between the abundance of Lactarius-type mycorrhizas and distance along the troughs. In contrast, there was no significant correlation between the mean percentages of Laccaria-type or Hebeloma-type mycorrhizas and distance along the troughs, the correlation coefficients in these cases being 0.686 and 0.319 respectively.

Interactions between inoculant fungi. The degree to which each of the inoculant-type fungi developed mycorrhizas in troughs where only one type of inoculum was present (troughs 21 to 24 for *Laccaria*, 25 to 28 for *Hebeloma*

Fig 4.4.5
Distribution of root attributable to *Lactarius* in each sampling position in October 1986 (means of 32 positions) with equation of line, correlation coefficient (r) and level of significance (P).



and 29 to 32 for *Lactarius*) was compared, by t-test, with the degree of development in troughs in which all three types had been applied to each sampling band (troughs 33 to 36 in Tables 4.4.12 - 4.4.14). The comparisons, shown below, are based on mean percentages of roots of each mycorrhizal type in a total of 28 sampling positions in four troughs.

Mean percentage of root tips with mycorrhizas of Laccaria, Hebeloma and Lactarius in troughs to which each fungus was added alone and in troughs in which all three fungi were added to all sampling positions.

Fungus	Single inoculum	Mixed inoculum	Significance of difference
Laccaria	1.61	0.39	P = 0.05
Hebeloma	14.58	19.33	P = nil
Lactarius	12.47	3.70	P = 0.001

There was no significant difference in the case of Hebeloma-type mycorrhizas, but the development of both Laccaria- and Lactarius-type mycorrhizas was significantly decreased in the presence of three inoculant fungi than in, respectively, the presence of *Laccaria* or *Lactarius* inoculum alone.

Distribution of mycorrhizas of other types. Tables 4.4.15 - 4.4.18 show the percentage of root tips attributable to mycorrhizas other than inoculant types in each sampling position of the 32 troughs. A total of five non-inoculant types developed on the root systems, presumably from naturally occurring inoculum in the soil or, in some instances, perhaps from inoculum introduced on the original sapling roots. These types were distinctive and were identified as belonging to *Thelephora*, *Inocybe*, *Cenococcum*, *Leccinum* and *Paxillus*. The distribution of mycorrhizas of these types between the seven sampling positions was subjected to analysis of variance, as shown in the table below, the data being for percentage of mycorrhizal tips, including zero values where appropriate.

Table 4.4.15
Percentage root tips attributable to Inocybe

Tree	Sampling position						
	1	2	3	4	5	6	7
9	21.3	15.9	13.3	21.6	11.9	15.4	10.4
10	1.5	0	11.7	0	16.3	7.9	0
11	13.3	16.1	10.9	20.5	0	12.2	6.0
12	12.3	12.4	7.3	1.5	0	3.6	3.9
13	2.3	11.2	1.9	0	26.2	13.0	15.7
14	0.9	5.6	8.4	8.5	5.0	15.5	9.6
15	9.1	5.0	4.0	16.0	22.5	20.5	17.5
16	13.3	18.1	13.8	12.6	5.4	26.7	9.6
17	23.7	12.3	23.9	12.8	25.4	34.7	49.0
18	11.7	18.6	6.3	21.1	5.9	4.4	20.0
19	10.0	8.7	30.5	15.4	22.7	18.7	21.4
20	0	9.7	13.7	13.9	9.6	6.9	8.1
21	3.7	9.6	16.9	27.4	21.7	31.4	11.4
22	10.5	25.9	12.7	15.7	5.6	0	4.6
23	0.2	3.5	6.2	5.0	3.0	0	0
24	13.0	34.0	19.6	8.4	13.7	15.9	13.8
25	15.1	15.0	31.1	3.5	44.9	3.5	16.6
26	1.5	4.6	7.9	20.6	14.6	26.2	22.8
27	4.0	8.2	8.3	26.8	12.6	4.4	13.2
28	24.2	16.0	25.4	20.2	36.7	25.7	27.6
29	0.7	2.6	0	1.7	4.3	1.9	6.5
30	8.1	5.9	1.2	2.8	14.1	8.1	6.5
31	9.7	8.6	7.9	32.2	22.2	10.6	12.4
32	2.0	13.1	8.2	5.8	11.8	4.7	13.7
33	24.9	14.2	13.9	11.3	14.1	15.1	13.9
34	18.1	27.7	27.9	28.2	14.2	30.8	29.3
35	0	0	0	0	0	0	0
36	4.5	13.4	5.3	0	11.8	2.1	8.0
37	0	2.3	15.1	28.4	20.0	27.8	20.5
38	5.5	10.3	10.0	29.1	8.8	6.0	33.1
39	12.1	14.3	17.0	16.5	21.0	27.8	20.5
40	11.2	10.7	12.1	17.3	2.1	2.9	12.3

Table 4.4.16
Percentage root tips attributable to Thelephora

Tree	Sampling position						
	1	2	3	4	5	6	7
9	6.7	8.3	7.9	17.3	1.9	5.6	18.0
10	15.1	27.3	26.7	62.1	44.8	40.6	27.0
11	32.3	29.1	33.8	53.4	63.2	59.5	47.2
12	18.0	8.7	22.2	29.2	18.5	26.1	27.5
13	18.2	19.9	44.0	22.0	16.0	36.6	35.8
14	16.8	10.7	13.8	11.2	18.3	5.1	15.5
15	18.1	15.7	25.2	8.7	28.9	19.9	21.8
16	9.2	13.7	7.9	0	2.7	21.7	15.3
17	30.1	23.9	16.3	14.6	21.8	7.2	22.7
18	19.9	26.0	24.8	30.0	23.7	14.5	13.6
19	8.4	18.0	30.5	15.4	22.7	18.7	21.4
20	6.5	6.3	5.3	1.8	1.7	2.8	9.4
21	13.3	18.4	8.9	3.8	12.7	6.3	31.6
22	26.8	12.4	8.7	0	21.6	25.7	41.0
23	29.8	18.6	14.0	29.0	18.3	25.9	7.0
24	17.6	16.1	16.4	34.7	14.4	29.5	23.6
25	36.0	28.3	43.8	59.4	29.7	19.0	27.4
26	13.0	17.1	20.6	30.0	17.7	19.1	35.3
27	20.3	22.1	48.6	29.3	28.7	35.2	28.8
28	18.5	25.3	31.6	27.4	14.3	12.3	34.8
29	3.5	7.6	10.9	13.1	6.9	8.3	17.0
30	23.9	20.4	22.5	35.1	37.1	24.1	37.1
31	13.6	0	31.1	0	50.3	52.5	75.2
32	24.6	24.2	22.5	35.1	37.1	24.1	37.1
33	18.2	24.0	37.4	42.8	21.1	17.0	10.8
34	45.0	33.1	37.3	20.4	11.0	52.1	48.7
35	0	7.3	45.3	2.8	3.2	2.7	15.4
36	20.4	20.3	19.2	29.1	3.6	6.4	6.0
37	20.7	40.7	24.3	12.9	23.0	21.5	14.5
38	18.1	17.8	13.2	35.8	39.3	31.8	26.9
39	17.1	22.4	35.0	59.0	49.7	47.2	31.4
40	24.1	18.5	19.8	26.9	41.8	32.7	37.0

Table 4.4.17

Percentage root tips attributable to Cenococcum

Tree	Sampling position						
	1	2	3	4	5	6	7
9	0	7.8	7.8	1.7	3.7	1.1	0
10	2.2	1.6	1.9	0	0	3.3	0
11	6.2	17.8	9.1	1.4	0	0	4.1
12	2.6	0	0	4.5	0	0	8.9
13	8.1	5.7	0	0	0	0	0
14	6.0	0	0	1.2	0	0	0
15	15.8	10.3	0	7.7	15.1	7.0	0
16	0	0	0	0	1.4	0	0
17	11.6	1.5	8.6	1.9	0	0	0
18	11.2	0	0	0	0	3.2	3.9
19	9.0	4.7	12.5	2.2	0	0	0
20	3.8	0	2.2	0	0	14.7	3.0
21	1.1	12.9	36.9	11.5	5.8	6.0	0
22	10.0	32.8	6.3	6.7	28.6	21.4	0
23	16.3	0.9	0	6.4	7.0	0	21.8
24	2.7	4.4	0	0	14.4	1.5	18.6
25	14.8	17.1	3.5	4.4	0	1.5	1.0
26	40.7	12.5	18.0	5.6	3.5	0	0
27	0	0	3.9	0	0	0	0
28	0	0	0	0	0	0	0
29	0	0	4.2	0	0.7	0	0
30	23.9	28.4	21.6	31.1	0	0	1.6
31	0	0	0	0	0	0	0
32	10.4	0	0	3.4	0	0	0
33	6.9	10.3	3.4	2.6	0	0	0
34	12.1	3.9	0	6.3	58.2	0	0
35	11.3	15.5	3.1	2.9	0	0	0
36	0	0	0	0	0	0	3.2
37	12.9	7.2	0.4	0	0	0	0
38	0	0	0	0	0	0	0
39	21.8	20.2	22.1	3.7	0	2.0	2.3
40	9.8	12.0	26.9	7.1	3.8	0	0

Table 4.4.18
Percentage root tips attributable to Paxillus / (Leccinum)

Tree	Sampling position						
	1	2	3	4	5	6	7
9	58.7	52.6	29.6	21.6	16.4	23.2	14.2
10	4.4	0	0	0	0	0	0
						(1.8)	
11	4.2	4.6	0	0	0	0	0
			(16.2)				
12	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0
15	1.4	0	0	0	0	0	0
	(17.0)	(14.9)					(6.1)
16	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0
18	0	0	0	0	13.0	24.2	18.0
19	0.8	0	0	0	0	0	0
20	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0
	(6.5)	(5.2)	(4.6)	(11.2)	(0.8)		
22	0	0.6	0	0	0	0	0
	(0.6)	(4.0)	(4.5)				
23	1.7	0	0	0	0	0	0
24	0	0	0	0	0	0	0
25	8.5	1.5	4.5	0	0	0	0
26	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0
28	1.4	0	0	0	0	0	0
29	0	0	0	0	0	0	0
30	0	1.4	5.8	0	0	0	0
31	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0
	(16.1)	(10.1)					
33	7.9	1.4	2.2	0	0	0	0
34	2.0	0	0	0	0	0	0
						(2.8)	
35	0	0	3.7	0	0	0	0
36	0	0	0	0	0	0	0
37	5.5	0	0	0	0	0	0
	(6.2)	(2.4)					
38	0	0	0	0	0	0	0
					(4.7)		
39	12.4	4.0	0	0	0	0	0
40	4.3	0	0	0	0	0	0

Fungus	1	2	3	4	5	6	7	SED	5% LSD	1% LSD
Thel	18.9	18.8	24.0	24.8	23.1	23.5	26.8	4.1	-	-
Inoc	9.0	11.7	12.3	13.9	14.6	2.4	4.8	-		
Cen	8.5	7.1	6.0	3.5	4.4	1.9	2.1	2.0	4.2	5.6
Pax	3.5	2.1	1.4	0.7	0.9	1.5	1.0	1.6	-	-
Lecc	1.4	1.1	0.8	0.3	0.2	0.1	0.2	0.6	-	-

(Thel = Thelephora ; Inoc = Inocybe ; Cen = Cenococcum
Pax = Paxillus ; Lecc = Leccinum
SED = standard error of the difference between any two means
LSD = least significant difference between any two means.)

From this table it is clear that *Thelephora* was the dominant non-inoculant mycorrhizal type in the experiment as a whole, followed by *Inocybe*, whereas relativaly small proportions of root tips were infected by *Cenococcum*, *Leccinum* and *Paxillus*. It should be mentioned also that at least some of the mycorrhizas of *Hebeloma*, discussed earlier, would have arisen from naturally occurring inoculum. The data above show that there was no significant difference in the development of either Thelephora- or Paxillus-type mycorrhizas at different positions in the troughs but there were significant differences in the distributions of the other three types. The incidence of both Cenococcum- and Leccinum-types decreased with increasing distance from the tree bases whereas the development of Inocybe-type increased with distance.

These relationships were anaylsed further, using a curve-fitting computer program ("Graphkit"), as shown in Figs 4.4.6 - 4.4.10. Then for each mycorrhizal type there was a significant relationship between frequency of mycorrhizas and distance along the trough.

The development of the three commonest non-mycorrhizal fungi, namely *Thelephora*, *Inocybe* and *Cenococcum*, was not significantly affected by

Fig 4.4.6 - 4.4.10
Distribution of root tips attributable to various mycorrhizal types in October 1986 in each sampling position (means of 32 positions) with equation of line, correlation coefficient (r) and level of significance (p).

Fig 4 4 6

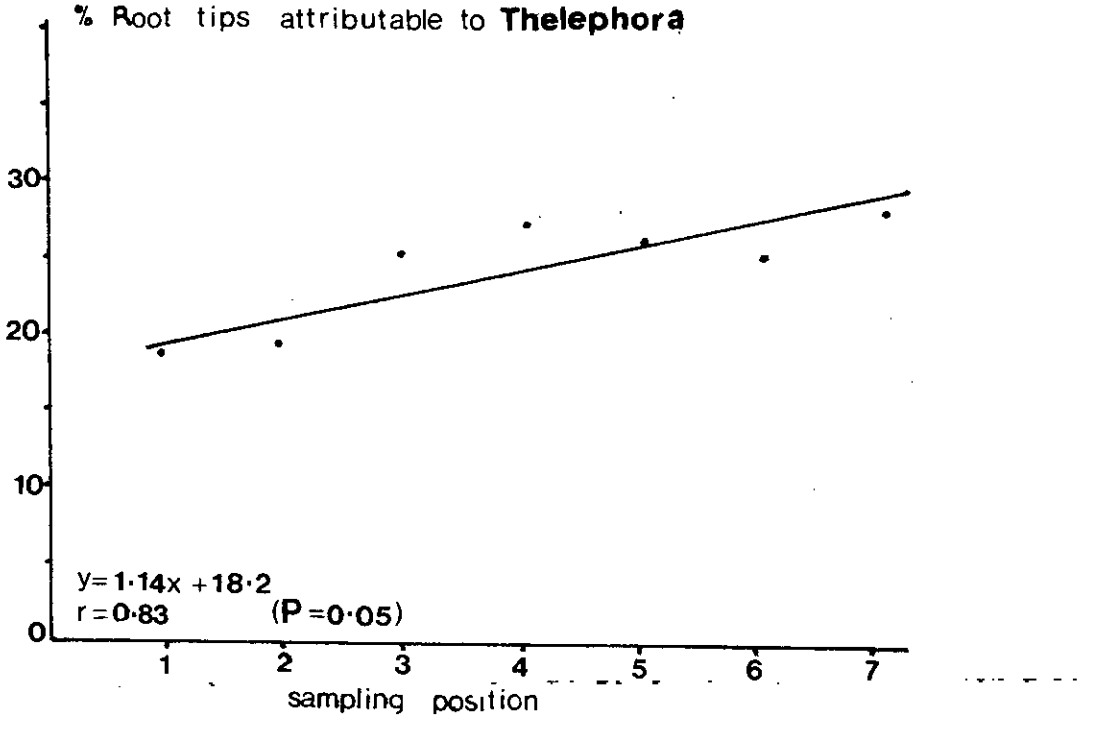


Fig 4 4 7

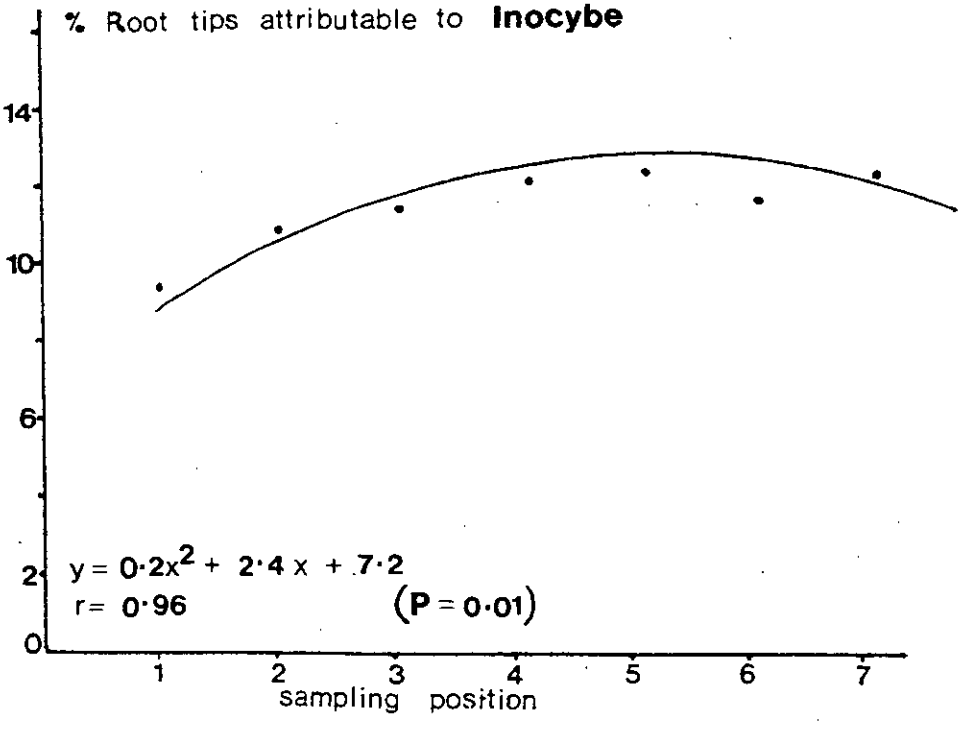


Fig 4.4.8

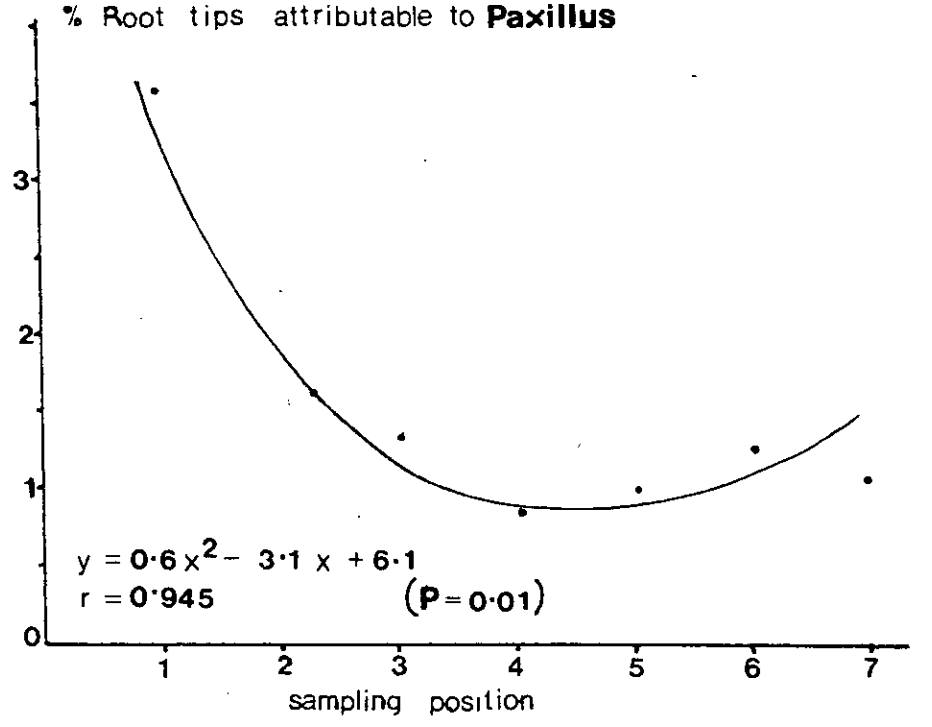


Fig 4.4.9

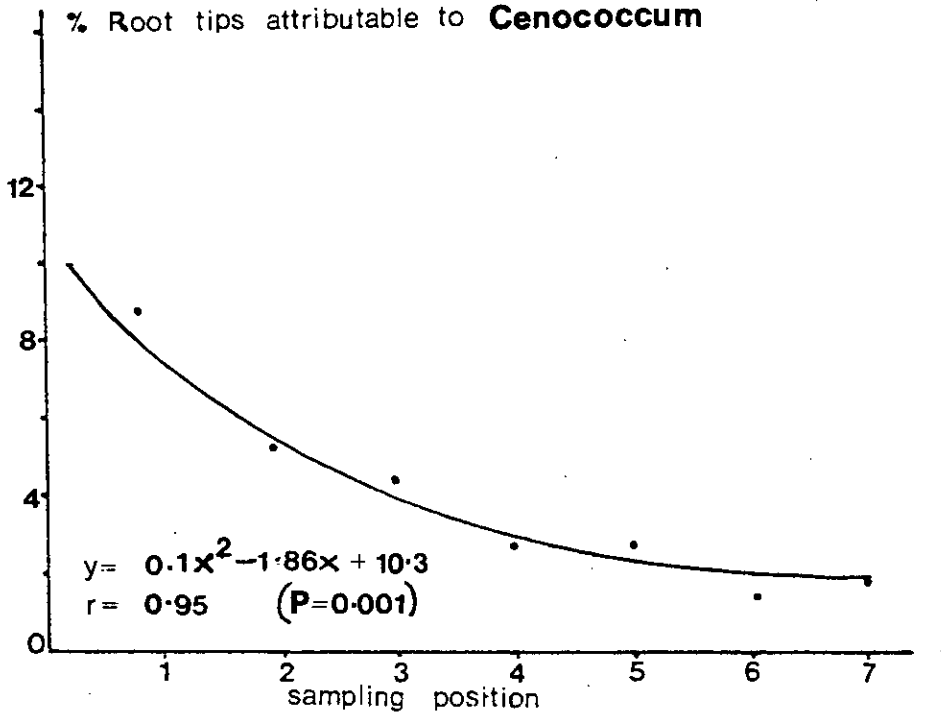
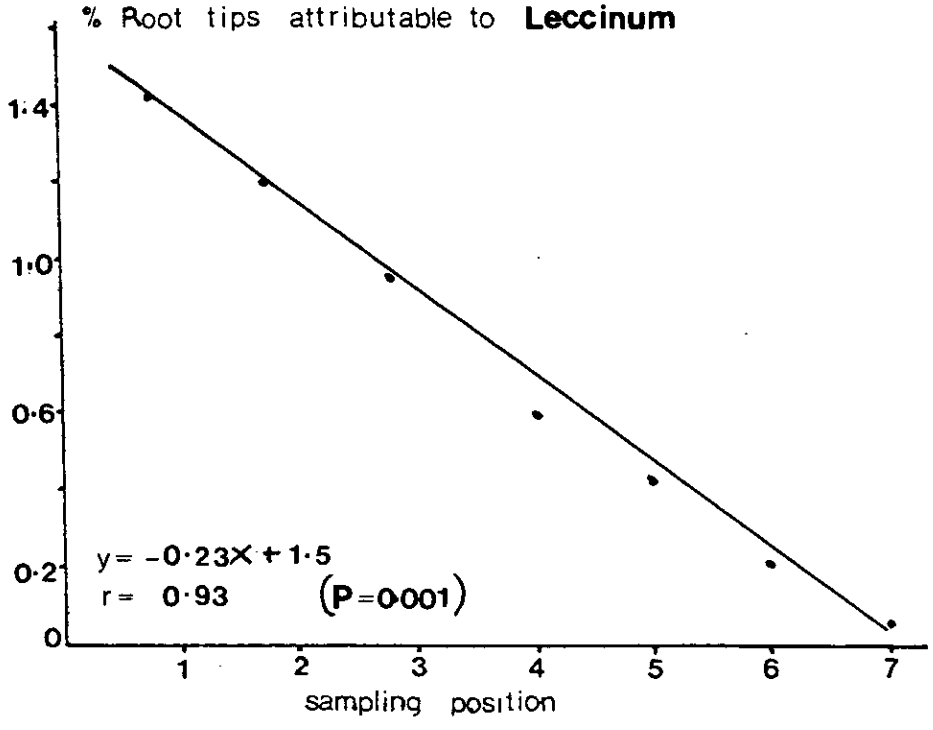


Fig 4.4.10


% Root tips attributable to **Leccinum**
1·4
1·0
0·6
0·2
0
y = -0·23X + 1·5
r = 0·93
(P=0·001)
1
2
3
4
5
6
7
sampling position

the presence or absence of inoculum of *Hebeloma* or *Lactarius* as shown in the table below, based in all sampling positions respectively with or without added inoculum of the appropriate fungus.

Mean percentage (+/- s.e.) of mycorrhizas attributable to Thelephora, Inocybe or Cenococcum in sampling positions to which inoculum of Hebeloma or Lactarius had or had not been added.

Fungus	+ Hebeloma	- Hebeloma
Thelephora	22.5 ± 1.5	23.1 ± 1.2
Inocybe	13.5 ± 1.1	12.2 ± 0.8
Cenococcum	4.5 ± 1.0	5.0 ± 0.7
	+ Lactarius	- Lactarius
Thelephora	21.8 ± 1.7	23.5 ± 1.1
Inocybe	11.3 ± 1.0	13.5 ± 0.8
Cenococcum	4.4 ± 0.9	5.1 ± 0.7

The mean percentage of moribund root tips (plus a small proportion of uninfected root tips) was likewise not significantly affected by the presence or absence of added inoculum; the mean percentages were 37.4% and 40.3% respectively with and without inoculum of *Hebeloma*, and 39.2% either in the presence or absence of *Lactarius*.

Development of fruitbodies. The types of fruitbodies that occurred in each trough in 1985 and 1986 and the position of each in relation to the positions of inoculation are shown in Table 4.4.19.

Inspection of the tables reveals several general points. In 1985, fruitbodies occurred predominantly in the first five sampling positions whereas in 1986 they were recorded along the lengths of the troughs; this difference doubtless reflects the fact that in the first season the root systems did not extend the entire lengths of the troughs, but after a further year's incubation they

Table 4.4.19 (a)
Summary of fruitbody development in troughs 9 - 40 in 1985.

Trough	Sampling position							Total
9	-	I/T	5I	-	-	-	-	7
10	-	-	-	-	-	-	-	0
11	-	-	-	-	-	-	-	0
12	-	-	-	-	-	-	-	0
13	-	-	T	-	-	-	-	0
14	-	2T	2I/2T	I	T	-	-	8
15	-	2I	4I	-	-	-	-	6
16	-	-	-	-	-	-	-	0
17	-	-	-	-	-	-	-	0
18	-	2I/T	2I	-	T	-	-	6
19	T	T	T	T	3T	-	-	7
20	-	T/L	T	-	-	-	-	3
21	-	-	-	-	-	-	-	0
22	T	4T	3T	T	-	-	-	9
23	-	2T	-	T	-	-	-	3
24	-	-	-	-	-	-	-	0
25	4I/T	2I/5T	2T	T	2T	T	-	18
26	T	T	-	-	2T	-	-	4
27	-	-	-	-	-	-	-	0
28	-	-	-	-	-	-	-	0
29	-	-	-	-	-	-	-	0
30	-	-	-	-	-	-	-	0
31	T	-	-	-	T	-	-	2
32	-	-	-	-	-	-	-	0
33	I	I	I	I/T	-	-	-	5
34	-	-	-	-	-	-	-	0
35	-	I/T	-	-	-	-	-	2
36	-	-	-	-	-	-	-	7
37	-	T	-	-	-	-	-	1
38	I	-	-	T	T	-	-	3
39	I/T	2I/3T	I/T	I/L	2T	-	-	13
40	-	-	-	-	-	-	-	0
Total	13	35	26	10	13	1	0	98

(-, no fruitbodies observed)

Table 4.4.19 continued.
Summary of fruitbody development in troughs 9-40 in 1986

Trough	Sampling position							Total
	1	2	3	4	5	6	7	
9	I/2T/P	2I/T	2I/T	I	-	3T	-	14
10	3I/2P	I	2I	I	-	-	-	9
11	-	-	2P	-	-	P	-	3
12	-	-	-	-	-	-	T	1
13	-	I/P	P	P	-	P	T/6P	12
14	-	I/T	-	T	4I	I	I/T	10
15	-	-	I	-	-	-	T	2
16	-	-	-	-	-	-	-	0
17	I/P	2I/2P	P	-	-	-	-	7
18	2P	-	3I	-	5T	-	-	10
19	T	-	-	-	-	-	-	1
20	-	-	-	-	-	-	-	0
21	-	-	T	I	2I	I	3I	8
22	3I/P	-	T/P	I	-	-	T	8
23	-	-	-	I	-	-	-	1
24	-	-	-	-	-	-	-	0
25	5I/2P	T	I	2T	-	I	2T/3P	17
26	-	-	-	-	-	-	2I/2T	4
27	-	I	I	-	-	-	-	2
28	-	-	I	-	-	2I	-	3
29	-	Lp	-	-	-	-	-	1
30	4H	-	3H	H	-	-	-	8
31	-	2I/2T	-	-	-	2I	-	6
32	-	H	-	-	-	-	-	1
33	-	I	-	I	I	-	2I/T	6
34	2H	2P	I	-	2P	2P	-	9
35	2H	2H	-	-	-	-	-	4
36	5H	-	-	-	-	-	-	5
37	-	-	-	-	-	-	6L	6
38	I	3I	I	H	-	3T	T	10
39	-	-	T	-	2I	-	2T	5
40	7H	4H	2H	H	-	-	-	14
Total	46	32	27	13	16	17	36	187

T = Thelephora ; I = Inocybe ; P = Peziza ; H = Hebeloma ;
L = Laccaria ; Lp = Lactarius/pubescens.

did so. Nevertheless, application of chi-squared analysis, as in the table below, shows that the total number of fruitbodies occurring in each sampling position of the 32 troughs differed significantly in both seasons. In 1986, for example, the fruitbodies were commonest in regions both closest and furthest from the tree base but were less common in the middle regions of the troughs. The reasons for this are not known.

Total number of fruitbodies recorded in each sampling position.

Year	1	2	3	4	5	6	7	Calc χ^2 *	Signif. of diff.!
1985	13	33	27	10	13	1	0	63.8	P=0.001
1986	46	23	27	13	16	17	36	33.2	P=0.001

(*, based on assumption of randomness of distribution
!, significance of difference.)

In 1985 three types of fruitbodies were observed in the troughs - mainly *Thelephora* and *Inocybe* fruitbodies but with one recorded fruitbody of *Laccaria*. However, in 1986 six types of fruitbody were recorded in the troughs; again *Thelephora* and *Inocybe* were commonest, but considerable numbers of *Hebeloma* and *Peziza* fruitbodies were seen, as were several fruitbodies of *Laccaria* in one trough and one fruitbody of *Lactarius pubescens* in another trough. The numbers of fruitbodies and their positions along the troughs in each year are summarised in Table 4.4.20.

The distribution of each type of fruitbody along the troughs was analysed by the chi-square test, with a null hypothesis that the distribution would be even. As shown in Table 4.4.20, whereas the distribution of *Thelephora* fruitbodies was uneven along the length of the troughs in both years, fruitbodies of *Inocybe* became evenly distributed in the second year. *Hebeloma* fruitbodies,

Table 4.4.20
Numbers of fruitbodies of mycorrhizal fungi observed in each sampling position (1-7) in 1985 and 1986.

1985

Fungus	Sampling position							Total	Calc X2	Significance of difference
	1	2	3	4	5	6	7			
Thel	6	22	12	7	13	1	0	61	40.4	P=0.001
Inoc	7	11	15	2	0	0	0	35	44.8	P=0.001
Lacc	0	0	0	1	0	0	0	1	n.d.	*

1986

Fungus	Sampling position							Total	Calc X2	Significance of difference
	1	2	3	4	5	6	7			
Thel	3	5	4	3	4	6	16	42	20.7	P=0.05
Inoc	14	14	14	6	9	7	5	69	10.0	P=nil
Lacc	0	0	0	0	0	0	6	6	n.d.	
Heb	20	7	5	3	0	0	0	35	32.0	P=0.001
Pez	9	5	4	1	2	4	9	34	0.3	P=nil
Lact	0	1	0	0	0	0	0	1	n.d.	

(*, not determined because insufficient fruitbodies were observed)

which first appeared in 1986, were unevenly distributed, but *Peziza* fruitbodies which also appeared first in 1986, were evenly distributed along the length of the troughs. When the numbers of fruitbodies at each end of the troughs (i.e. in positions 1 - 3 as opposed to positions 5 - 7) in 1986 were compared by chi-square test (detailed below) it was found that significantly more fruitbodies of *Hebeloma* occurred in the first three sampling positions than in the last three, whereas numbers of *Peziza* fruitbodies were not significantly different between the ends of the troughs. The numbers of fruitbodies of *Thelephora*, which overall had a significantly uneven distribution when analysed for individual sampling positions, were also significantly different between positions 1 -3 and 5 -7 respectively in 1986. Also, significantly more fruitbodies of *Inocybe* occurred in positions 1 - 3 than in 5 -7 , although their overall distribution was not significantly uneven when assessed on the basis of individual sampling positions.

Numbers of fruitbodies occurring at each end (sampling positions 1 - 3 or 5 - 7) of the 32 troughs in 1986.

Fungus	1 - 3	5 - 7	Calculated χ^2	Significance of difference
Thelephora	12	27	5.0	P = 0.05
Inocybe	42	21	7.0	P = 0.01
Hebeloma	32	0	32.0	P = 0.001
Peziza	18	15	0.3	P = nil

A clear pattern thus emerges from the combined data for positions 1 - 3 and 5 - 7 respectively in 1986. Fruitbodies of *Inocybe* and *Hebeloma* were markedly more common in regions of the troughs closer compared with further from the tree bases, whereas there was a tendency for fruitbodies of *Thelephora* to occur further from the tree bases (in particular, in the furthest regions of the troughs). Fruitbodies of *Peziza*, however, were quite uniformly distributed along

the troughs.

Further inspection of Table 4.4.19. reveals that only three troughs (11, 20 and 24) did not contain fruitbodies of one type or another in 1986. Moreover, only 4 of the 32 troughs (11, 16, 20 and 24) did not bear fruitbodies of mycorrhizal fungi in this season, assuming that *Peziza* was not a mycorrhizal symbiont. Three of these four troughs (11, 16 and 24) also had not developed fruitbodies in the 1985 season. There is no obvious reason why these four troughs should not have developed fruitbodies, because they contained numerous mycorrhizas of at least Thelephora-type and, in most instances, of Inocybe- and Hebeloma-type. Similarly, there is no obvious reason why *Hebeloma* fruitbodies should have developed in only 7 of the 32 troughs (troughs 30, 32, 34, 35, 36, 38 and 40) (Table 4.4.19); comparison of this table with Table 4.4.13 shows that the positions where *Hebeloma* fruitbodies occurred did not necessarily correspond to positions where mycorrhizas of *Hebeloma* predominated, and there were some other troughs (eg numbers 16 and 26) that did not contain *Hebeloma* fruitbodies and yet in which Hebeloma-type mycorrhizas were as common as in troughs in which fruitbodies of *Hebeloma* occurred. In fact, overall, there was no clear relationship between the occurence of fruitbodies of the commonest types and the predominance of mycorrhizas in the troughs, as shown in the table below, where the mean percentage of mycorrhizas of *Hebeloma*, *Inocybe* or *Thelephora* is calculated for all positions in which fruitbodies of these fungi occurred and similarly for all positions in which fruitbodies of the respective fungi were not observed.

Mean percentage of mycorrhizal tips of the appropriate fungus in positions in which fruitbodies were and were not observed.

Fungus	With fruitbodies	No fruitbodies	SED	Significance of difference
Thelephora	26.4	22.4	3.0	n.s.d
Inocybe	14.8	12.2	1.4	n.s.d
Hebeloma	19.8	12.6	4.4	n.s.d

(SED = standard error of the difference between any two means
n.s.d = no significant difference.)

In the case of no single fungus was there a significant difference in the mean percentage of mycorrhizas in positions respectively with and without fruitbodies, although in each case the mean percentage of mycorrhizas was somewhat larger in positions where fruitbodies occurred. In all of the 13 sampling positions in which *Hebeloma* fruitbodies were observed this was the only fruitbody type and similarly was the only type in 5 of the 7 troughs in which *Hebeloma* fruited. Neither the numbers of root tips in these 13 sampling positions nor the numbers in the 7 troughs was significantly different from the numbers in the other sampling positions of the troughs respectively. The reason for the unusual distribution of *Hebeloma* fruitbodies is thus unclear.

The times of the appearance of fruitbodies in the course of the experiment are shown in Figs 4.4.21 (for 1985) and 4.4.22 (for 1986). In both years fruiting occurred throughout June - October and lasted for a total of 13 weeks (weeks 25 - 39), but fruitbodies of different species tended to occur in "flushes".

Inocybe spp. tended to fruit intermittently throughout July, August and September of 1985, with the main flush of 23 fruitbodies occurring in weeks 30 - 33 (Table 4.4.21). In 1986 (Table 4.4.22) the main flush (50 fruitbodies) of *Inocybe* again occurred in weeks 30 - 34, but in this year an earlier smaller flush of 15 fruitbodies was observed in weeks 25 - 26.

Table 4.4.21
Record of fruitbody appearance in troughs 9 - 40
in 1985.

Fungus	Wk 25 23-29 June	Wk 26 30-6 Jun/Jul	Wk 27 7-13	Wk 28 14-20	Wk 29 21-27	Wk 30 28-3 Jul/Aug	Wk 31 4-10	Wk 32 11-17
Inoc	0	0	5	0	0	4	2	7
Thel	0	0	2	0	0	6	0	5
Lacc	0	0	0	0	0	0	0	0

Wk 33 18-24	Wk 34 25-31	Wk 35 1-7 Sept	Wk 36 8-14	Wk 37 15-21	Wk 38 22-28	Wk 39 29-5 Sept Oct	Total
10	0	0	0	0	0	1	35
20	8	0	0	1	4	15	61
1	0	0	0	0	0	0	1

Table 4.4.22
Record of fruitbody appearance in troughs 9 - 40
in 1986.

Fungus	Wk 25 22-28 June	Wk 26 29-5 Jun	Wk 27 6-12 July	Wk 28 13-19	Wk 29 20-27	Wk 30 27-2 Jul	Wk 31 3-9 Aug	Wk 32 10-16
Inoc	6	10	0	0	11	14	9	9
Thel	1	2	0	0	7	8	11	7
Lacc	0	0	0	0	0	0	0	0
Heb	0	0	0	0	0	1	3	15
Pez	0	0	0	0	1	0	8	15
Lact	0	0	0	0	0	0	0	0

Wk 33 17-23	Wk 34 24-30	Wk 35 31-6 Aug	Wk 36 7-13 Sept	Wk 37 14-20	Wk 38 21-27	Wk 39 28-4 SeptOct	Total
6	3	0	0	1	0	0	69
6	0	0	0	0	0	0	42
0	0	0	0	6	0	0	6
14	8	0	0	6	0	0	35
5	1	0	0	4	0	0	34
0	0	0	0	0	0	1	1

Fruiting of *Thelephora* tended to occur in two large flushes in 1985 (39 fruitbodies in weeks 30 - 34 and 20 fruitbodies in weeks 37 - 39), but in the following year only the earlier flush (31 fruitbodies in weeks 29 - 33) was observed.

Only one fruitbody of *Laccaria* was produced in 1985 and this occurred in week 33. In 1986 there were 6 fruitbodies of *Laccaria* but these were produced simultaneously - and in close proximity - in week 37.

In 1986 6 species of fungi fruited in the 32 troughs that remained, compared with 3 species in 1985. One fruitbody of *Lactarius pubescens* was observed in week 39 but there were also numerous fruitbodies of *Hebeloma* and *Peziza*, both of which tended to fruit mainly between weeks 20 and 34 (26 fruitbodies of *Hebeloma* and 15 fruitbodies of *Peziza*) but 6 and 4 fruitbodies of *Hebeloma* and *Peziza* respectively were also produced in week 37.

Survival and distribution of seedlings

Of the 224 seedlings inserted into the troughs in 1985, only 40 survived until 1986. Only 33 seedlings had to be replaced within the first 4 weeks; the greatest losses occurred during the winter of 1985, when most seedlings did not regrow after dormancy.

Although the distribution of the 40 surviving seedlings between the seven sampling positions was not significantly uneven when analysed by chi-square test (see below) it is notable that almost half of the surviving 40 seedlings were in the two sampling positions closest to the tree base.

Numbers of seedlings planted in 1985 (in central position in troughs) and 1986 (down sides of troughs) that survived until October 1986.

Year	1	2	3	4	5	6	7	Calc χ^2	Signif. of diff.
1985	12	7	5	7	2	5	2	10.1	n.s.d
1986	6	4	4	4	2	4	4	2.0	n.s.d

(, based on assumption of randomness of distribution
, no significant difference.)

The seedlings that were inserted down the sides of the troughs were evenly distributed between the seven sampling positions owing to the selection of sites where they were planted. The four such seedlings that died were at positions 3,5 and 7. Overall, 68 seedlings were available for analysis when the troughs were sampled in October 1986.

Development of mycorrhizas

The root systems of the seedlings - both those in the middle and those down the sides - were small (mean 133 tips per plant) and there was no significant difference between the size of the retrieved root systems of seedlings from the middle and side positions, although those of the latter group tended to be larger (mean values 118 and 154 tips per plant respectively). The degree to which the root systems became mycorrhizal also tended to be greater for the seedlings in the side positions (mean 80.9% of root tips infected) than for those in the middle positions (mean 62.7%), a difference significant at $P < 0.01$ when analysed by Student's t-test. These results are somewhat surprising as the smaller, less mycorrhizal seedlings were approximately one year older than the others, but their shoot systems were usually very small and this reflected the smallness of their root systems.

The range of mycorrhizal types that developed on the seedling roots was similar to that which occurred on the older tree roots, as shown in Tables 4.4.23 and 4.4.24 and summarized in the table below. Of the types observed on the older tree roots, only *Leccinum* did not develop in the seedling root systems. Also the positions of the seedlings - whether in the middle or sides of the troughs - did not affect the development of the different mycorrhizal types.

Numbers of seedlings, which were situated in either middle or side positions in troughs, that bore mycorrhizas of each of the seven types.

Mycorrhizal type	Middle position	Side position	Total) (max 68)
Laccaria	6	8	14
Hebeloma	20	16	36
Lactarius	9	7	16
Thelephora	35	24	59
Inocybe	17	13	30
Cenococcum	7	5	12
Paxillus	0	2	2

The percentages of the root systems attributable to each mycorrhizal type are shown in Tables 4.4.23 and 4.4.24. The seedlings fell into two almost equal groups based on the presence or absence of Hebeloma-type mycorrhizas (53% and 47% of the seedlings respectively), so the development of each mycorrhizal type was examined overall and within these two groups.

Development of Thelephora-type mycorrhizas on seedling roots. By far the commonest mycorrhizal type overall, on seedlings as on the older trees, was Thelephora-type which accounted for approximately 34 percent of the seedling root tips. This was the only mycorrhizal type for which there was a significant difference in percentage occurrence on seedlings that, respectively, did or did not bear mycorrizas of *Hebeloma*, as shown below.

Table 4·4·23
Percentage of seedling roots attributable to observed mycorrhizal types for seedlings that bore mycorrhizas of Hebeloma.

Trough/ position *	Heb	Inoc	Thel	Lact	Lacc	Cen/Pax	Total myco
11/1	3.9	0	41.2	20.3	9.1	0/0	74.5
9/b4	64.0	2.7	21.1	0	0	0/0	87.8
10/b7	9.8	0	48.4	14.7	0	0/0	94.2
12/1	14.0	0	37.2	0	0	0/0	51.2
12/4	1.0	4.8	30.2	0	0	0/0	36.0
13/1	38.3	0	36.2	0	0	0/0	74.5
16/1	23.9	10.1	38.8	13.8	0	0/0	86.6
14/1	55.3	0	0	0	0	0/0	55.3
14/2	8.9	0	26.7	0	0	0/0	35.6
14/b2	72.4	0	25.0	0	0	0/0	97.4
14/b6	47.5	0	41.5	0	0	0/0	89.0
16/1	23.9	10.1	38.8	13.8	0	0/0	86.6
19/2	37.9	20.0	4.2	0	0	8.4/0	70.5
20/b2	30.2	1.1	50.0	0	18.7	0/0	100.0
21/b1	30.7	36.0	8.0	0	15.3	0/10.0	100.0
23/3	28.3	0	18.9	0	7.1	0/0	100.0
23/4	66.9	0	0	0	0	3.6/0	70.5

Table 4.4.23 continued

23/b1	49.5	0	16.8	0	0	0/0	66.3
23/b7	63.8	0	0	0	0	0/0	63.8
25/b1	5.4	4.0	65.8	0	0	0/0	75.2
26/b4	40.1	0	39.2	0	0	0/0	79.3
26/1	5.2	7.8	14.7	0	0	0/0	27.7
26/5	32.7	12.5	16.6	0	0	0/0	61.8
26/6	28.7	25.0	0	0	0	0/0	53.7
27/b7	61.6	0	33.3	0	0	0/0	94.9
28/b6	37.4	32.5	0	0	0	0/0	69.9
30/b4	1.0	1.0	67.3	2.0	0	9.4/0	80.7
32/1	13.6	9.3	51.2	0	0	8.8/0	82.9
32/b1	21.7	0	17.3	25.4	0	5.6/0	70.0
34/1	15.4	33.3	40.2	0	0	4.3/0	93.2
34/2	19.0	35.0	45.9	0	0	0/0	100.0
35/2	42.5	0	16.3	21.3	0	0/0	80.1
35/b2	18.6	0	0	33.6	0	0/7.1	59.3
35/b3	48.8	0	0	40.5	0	0/0	89.3
36/6	18.2	12.1	37.4	0	0	0/0	67.7
36/7	26.7	19.8	0	0	0	0/0	46.5
40/6	25.0	0	44.0	0	0	0/0	69.0
Mean	27.1	7.3	24.9	4.8	2.0	1.1/0.5	72.5

Table 4.4.24
Percentage roots attributable to observed mycorrhizal types for seedlings that did not bear mycorrhizas of Hebeloma.

Tree/ position	Inoc	Thel	Lact	Lacc	Cen	Total myco
9/b1	20.3	69.4	0	0	0	89.7
10/5	0	66.2	22.5	0	0	88.7
12/2	0	35.2	0	3.7	0	38.9
12/3	0	33.3	0	0	0	33.3
12/6	0	37.5	0	0	0	37.5
13/4	0	52.2	26.7	0	0	78.9
13/3	0	40.0	0	0	0	40.0
13/7	14.0	83.2	0	0	0	97.2
15/4	41.0	36.0	0	0	5.1	82.1
16.2	0	27.2	20.5	0	0	47.7
16/4	43.8	0	3.5	0	0	47.3
17/2	6.2	38.3	0	4.9	0	49.4
17/b3	17.8	66.4	0	0	4.0	88.2
17/b6	46.4	51.6	2.0	0	0	100.0
18/4	0	80.0	0	0	0	80.0
21/b7	13.6	33.7	0	15.4	0	62.7
23/1	0	33.3	0	18.0	21.7	73.0

Table 4.4.24 continued

24/b4	0	30.3	0	18.4	0	48.7
24/b5	28.4	50.0	0	10.8	0	89.2
25/1	0	89.5	0	0	0	89.5
26/b5	23.3	57.2	0	0	0	80.6
27/b3	0	88.8	0	0	9.4	98.2
28/3	0	30.7	0	9.1	0	39.8
30/b6	18.1	73.3	0	0	2.9	94.3
31/1	0	22.2	32.1	0	0	54.3
31/3	0	32.5	7.0	0	0	39.5
31/b1	0	20.8	75.3	0	0	96.1
33/b1	0	25.9	0	12.2	0	38.1
33/b3	0	54.8	0	5.7	0	60.5
33/4	0	31.5	37.9	0	0	69.4
34/6	33.3	11.1	0	0	0	44.4
40/1	29.4	6.1	0	0	49.6	85.1
Mean	10.5	43.9	7.1	3.1	2.9	67.6

Mean percentages of seedling root tips attributable to each mycorrhizal type on seedlings that did or did not bear mycorrhizas of Hebeloma.

Fungus	+ Hebeloma	-Hebeloma	SED	0.1% LSD
Laccaria	2.0	3.1	1.56	-
Lactarius	4.8	7.1	2.59	-
Thelephora	24.9	43.9	0.65	2.37
Inocybe	7.3	10.5	1.61	-
Cenococcum	1.1	2.9	2.15	-

(SED = standard error of the difference between any two means
-, no significant difference at this level.)

In each of the 59 instances in which seedlings developed mycorrhizas of *Thelephora*, the older tree also bore mycorrhizas of this type in the equivalent sampling position. But in 48 of these 59 seedlings the percentage of mycorrhizal tips attributable to *Thelephora* was greater on the seedlings than on the older tree. The differences between the percentage of Thelephora-type on seedlings and tree roots in equivalent positions was significant (P=0.001) when compared in a Paired samples t-test (Table 4.4.25).

There was no obvious reason why eight seedlings did not develop mycorrhizas of *Thelephora*, because in each case the older tree roots bore mycorrhizas of this type in equivalent positions and to a degree that was not significantly different from that in the other positions (mean 17.9 + 2.3% and 22.8 + 0.9% of the total root tips respectively). These eight seedlings were distributed between seven troughs and in each of these there was at least one other seedling that was infected by *Thelephora*.

Development of Hebeloma-type mycorrhizas on seedling roots. As with *Thelephora*, many of the seedlings bearing mycorrhizas of *Hebeloma* were positioned close to regions of Hebeloma-type development on the older tree

Table 4.4.25
Comparison of the occurrence of different mycorrhizal types on seedlings and on older tree root systems in equivalent positions (Paired t-test).

Myco type	Mean % occurrence in Tree	Seedling	No.of seedlings	Mean z *	Calc t	Signif of diff.
Lacc	3.72	6.56	19	4.37	2.80	P=0.05
Heb	16.10	20.11	52	9.58	3.66	P=0.001
Lact	11.53	12.56	26	5.97	2.30	P=0.05
Thel	22.80	34.46	67	16.65	6.99	P=0.001
Inoc	12.18	10.21	62	1.97	1.22	n.s.d.
Cen	4.91	8.73	27	3.80	1.77	n.s.d.

* z= difference between means

roots, but on sixteen seedlings *Hebeloma* failed to become established and yet was present on the older tree roots, and on four seedlings *Hebeloma* developed on the seedling roots but was not evident on the older tree roots.

In the sixteen cases in which *Hebeloma* did not develop on the seedlings despite establishment on the older tree roots, the degree of its establishment on the older tree roots (mean 9.8 ± 2.3%) was significantly (P=0.01) less than in positions where both seedling and older tree were infected with *Hebeloma* (mean 23.3 ± 2.9%).

In the four instances in which *Hebeloma* did develop on the seedlings but was absent from equivalent positions in the older tree it was found that the mean percentage of seedling roots attributable to *Hebeloma* was significantly (P=0.001) lower than when *Hebeloma* was also established on the older tree roots (means 11.1 ± 5.0% and 33.2 ± 3.5% respectively). Thus it seems that the development of mycorrhizas of *Hebeloma* on the seedling roots was influenced markedly by the presence of established mycorrhizas on the older tree root system.

There were 52 instances overall in which either the seedling or the older tree had mycorrhizas of *Hebeloma* (32 and 16 instances respectively). Within this overall grouping the seedling mean (20.1 %) and the older tree mean (16.1 %) did not differ significantly when compared by Student's t-test, but application of a Paired samples t-test, in which overall effects are compared irrespective of variations between individual trees, shows that the difference was highly significant (Table 4.4.25).

Development of Lactarius-type mycorrhizas on seedling roots. Sixteen seedlings (out of 68) were observed to have mycorrhizas of *Lactarius*. In 13 of

these instances the older tree roots also bore mycorrhizas of *Lactarius* but on 3 seedlings *Lactarius* became established in the absence of development on the older tree. One of these seedlings (beside sampling position 6 in trough 24) was close to *Lactarius* mycorrhizas in the trough and may have become infected by mycelial strands. The other two seedlings were growing in the same "low-Lactarius" trough (trough 10 with less than 1% Lactarius-type) but each seedling had a moderate percentage infection by *Lactarius* (22.5 % and 14.7 %).

Ten seedlings did not develop mycorrhizas of *Lactarius* even though this type was present on the older tree in equivalent positions. However, the proportion of the root tips attributable to *Lactarius* in these positions (mean 6.15%) was significantly (P=0.02) lower than in positions where the seedlings did develop *Lactarius* (mean 21.1%).

Overall, there were 26 instances in which either the seedling or the older tree or both had mycorrhizas of *Lactarius* (3,10,and 13 instances respectively) and in a Paired samples test (Table 4.4.25) there was a sig nificant difference between the development of *Lactarius* on seedlings and older tree roots (t=2.3) although the overall means (12.56% on seedlings and 11.5% on older tree roots) were not significantly different when compared in a Student's t-test (t=0.37).

Development of Inocybe-type mycorrhizas on seedling roots. Most (62 of the total 68) seedlings that survived until October 1986 were in sampling positions in which the older tree bore mycorrhizas attributable to *Inocybe*. *Inocybe* became established on the roots of half (31) of the seedlings but not on the other half. The mean percentages of *Inocybe* infection on the older roots in these two groups of sampling positions were not significantly different when analysed by Student's t-test (means 14.1% and 10.25% respectively) (t=1.89) so

the reason for the poor ability of *Inocybe* to establish on seedlings is unclear.

Where *Inocybe* did occur on seedlings, on the older tree roots or on both the mean percentage of roots attributable to *Inocybe* was not significantly different for seedlings (mean 10.21%) and the older tree (mean 12.18%) when analysed by a Paired samples t-test (t=2.11) (Table 4.4.25).

Development of Cenococcum-type mycorrhizas on seedling roots. The establishment of *Cenococcum* followed a similar pattern to that of *Inocybe*. Seedlings survived in 31 positions in which Cenococcum-type mycorrhizas occurred on the older tree root. Twelve of these seedlings were infected with *Cenococcum*, but the proportion of *Cenococcum* infection on the older roots near these twelve seedlings was not significantly different from that in the other fifteen positions in which seedlings did not become infected (12.5 ± 2.0% and 7.9 ± 1.5% respectively) (t=0.99). In the twelve instances when Cenococcum occurred on both seedling and tree roots, the mean percentage of root tips attributable to *Cenococcum* on seedling roots (mean 11.0%) was not significantly different from the mean percentage on the older tree roots (mean 10.3%) (t=0.32).

Development of Laccaria-type mycorrhizas on seedling roots. There were only nine instances in which mycorrhizas of *Laccaria* were present on seedlings and the older tree roots in equivalent positions. The percentage of root tips attributable to *Laccaria* on the seedlings (mean 12.6%) was not significantly different from the percentage on the older tree root systems (mean 6.3%) when compared in Student's t-test (t=2.04).

Four seedlings became mycorrhizal with *Laccaria* in the absence of *Laccaria* development on the older tree root system, and six seedlings failed to become infected with *Laccaria* when this mycorrhizal type was present on the

older tree root system (at a mean of 2.3%). However, when the occurrence of *Laccaria* on seedlings and corresponding older tree roots was compared in a Paired samples test the difference was significant at the 5 % level (t=2.8)(Table 4.4.25).

Development of Paxillus-type mycorrhizas on seedling roots. Neither of the two seedlings that became mycorrhizal with *Paxillus* was in a position where *Paxillus* had developed on the older roots, although in one instance *Paxillus* was present on the older tree in a nearby sampling position. In the other instance *Paxillus* was absent altogether from the older root system of the trough. In eleven other instances *Paxillus* did not establish mycorrhizas on seedling roots although it was present (at a mean 11.7%) on the other tree roots in equivalent positions.

Maps of mycorrhizal distribution on the lower soil surface.

The distribution of mycorrhizas visible to the naked eye on the bottoms of the troughs was recorded periodically as described in Section 4.2.3. The observed patterns on each of five occasions are shown in Figs 4.4.11 - 4.4.42.

The mycorrhizal types that were observed and were identifiable belonged mainly to *Hebeloma* and *Lactarius*, although mycorrhizas of *Leccinum* and *Paxillus* also were seen in some instances and were sufficiently distinctive to be identified. Of all these types, *Hebeloma* was the most conspicuous and readily identifiable because of the white appearance of the mycorrhizas and their associated hyphae, so most confidence can be placed in the records for this mycorrhizal type. Lactarius-type mycorrhizas were somewhat less distinctive and might have been confused at times with Thelephora-type mycorrhizas, unless the

FIG 4411 TROUGH 9

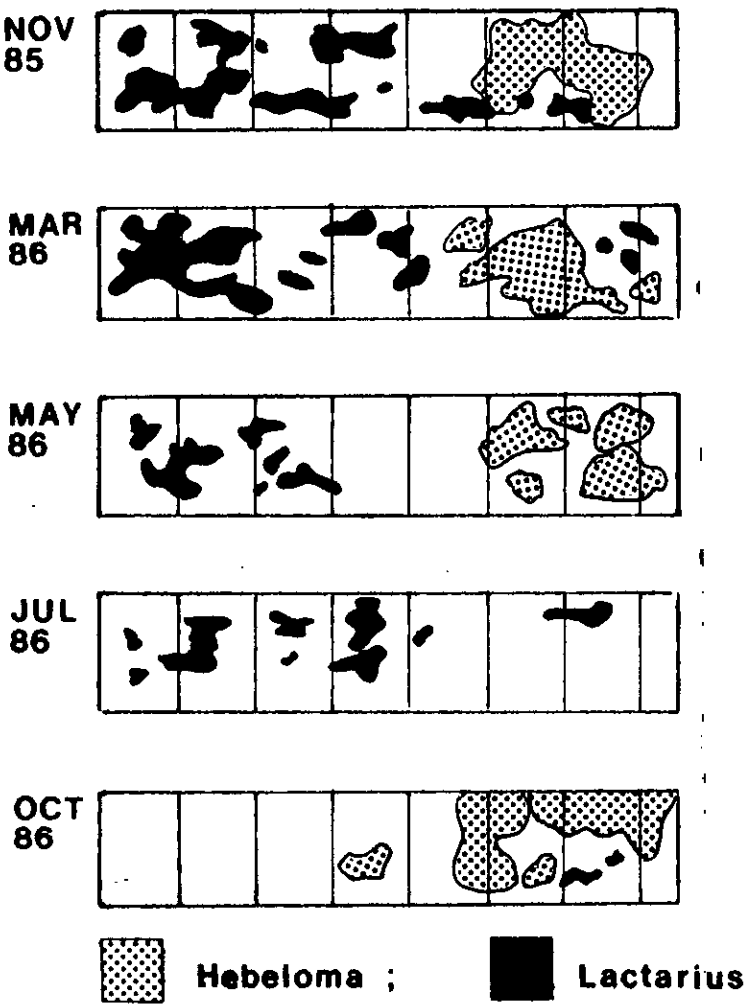
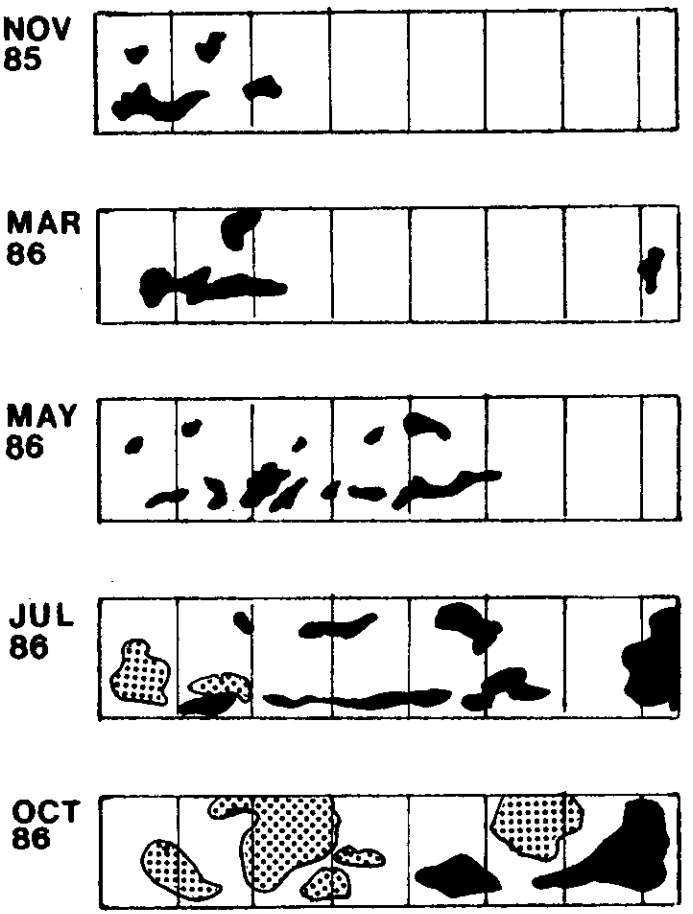


FIG 4412 TROUGH 10



Figs 4.4.11 – 4.4.42
"Maps" of distribution of mycorrhizal types visible on the lower surface of troughs 9 – 40, each containing one birch sapling.

FIG 44 13 TROUGH 11

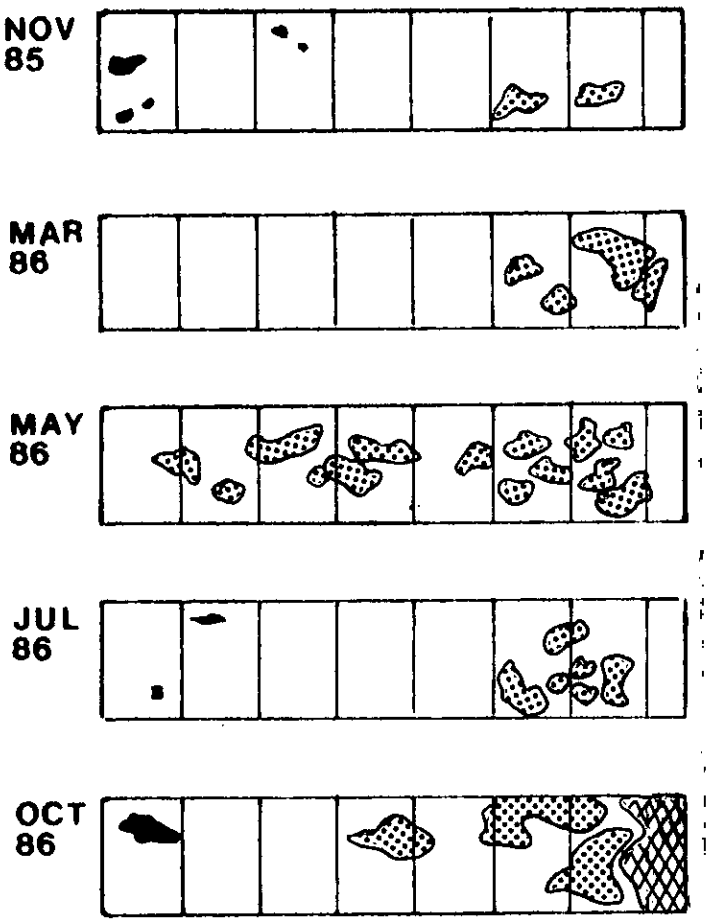
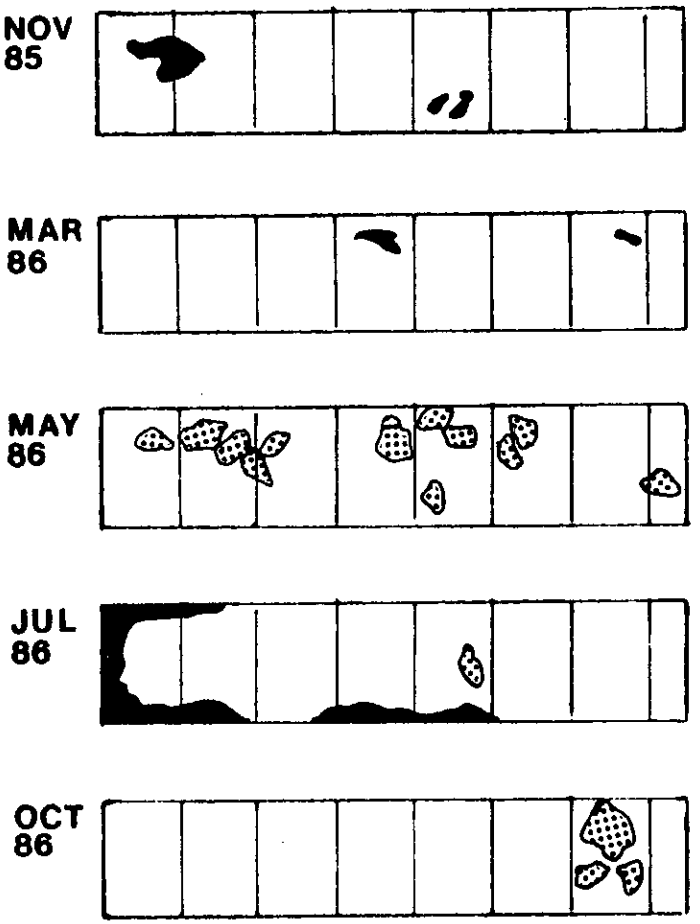


FIG 44 14 TROUGH 12



Hebeloma ; Lactarius ; Thelephora

FIG4415 TROUGH 13

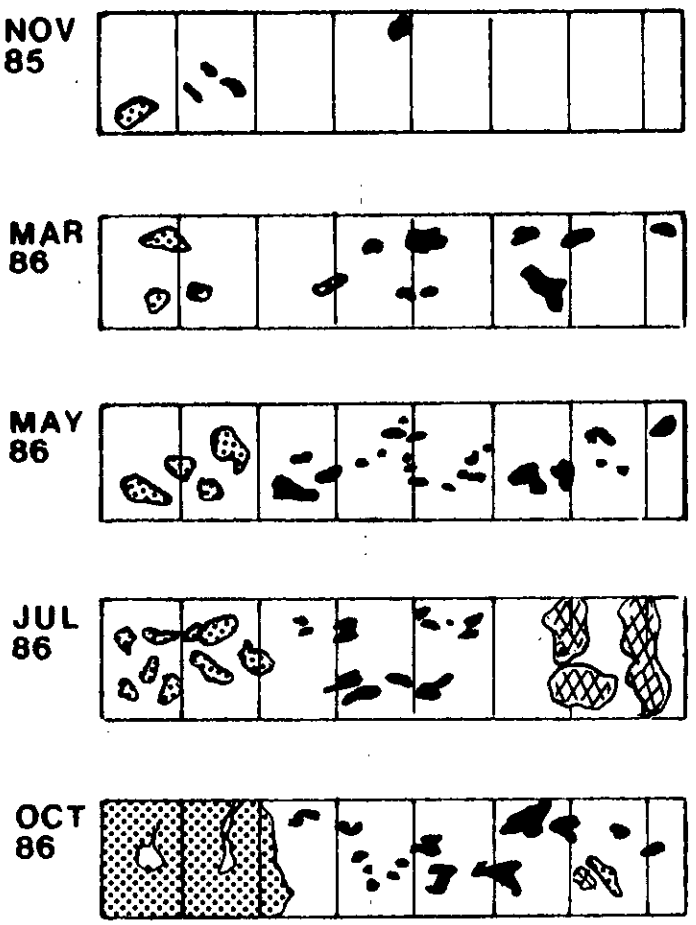


FIG4416 TROUGH 14

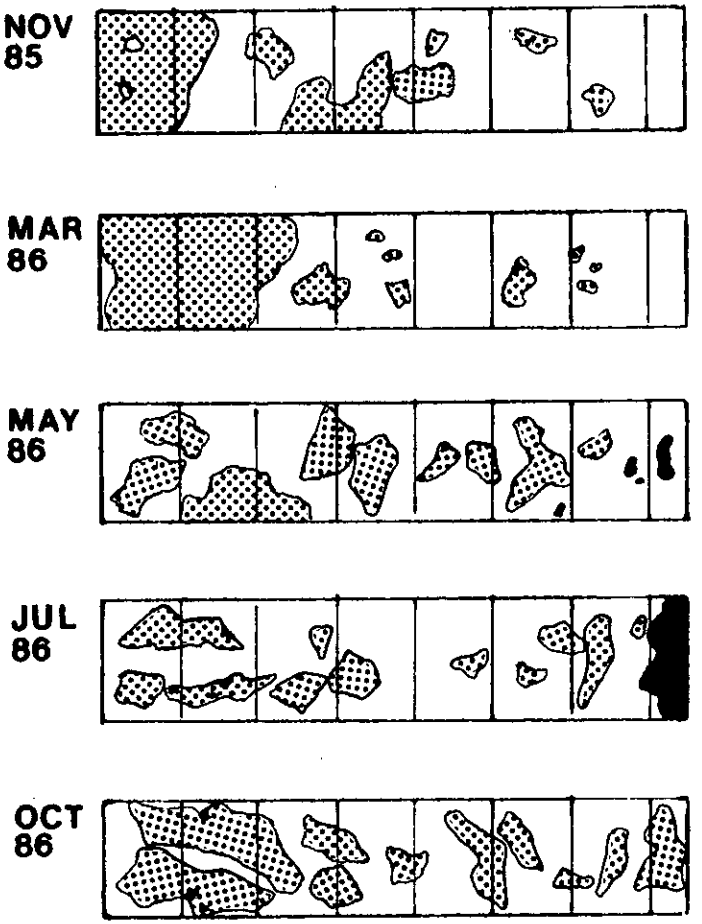


FIG 44 17 TROUGH 15

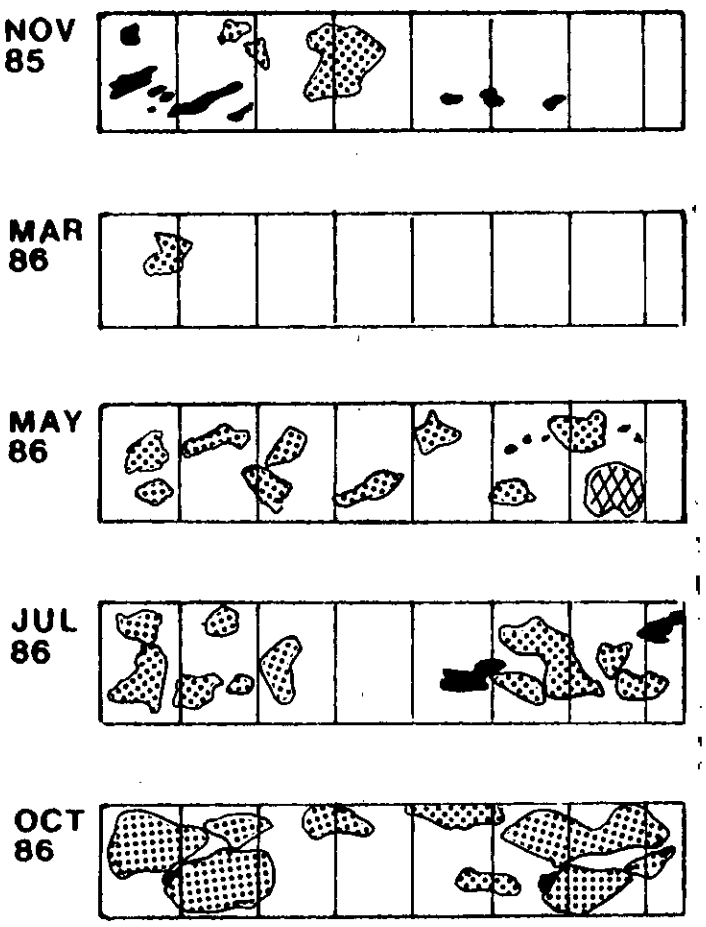
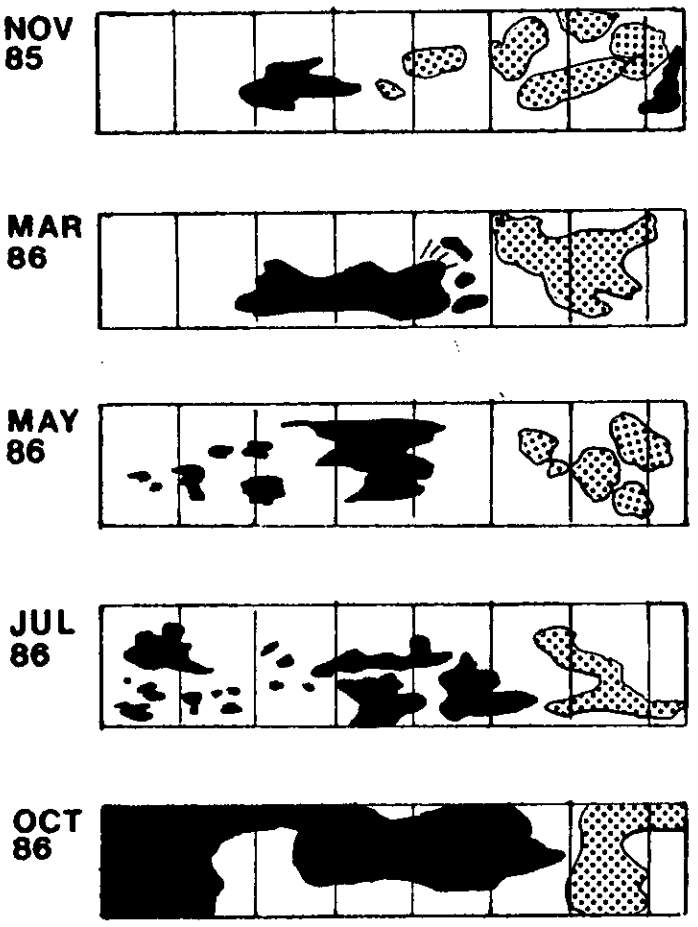


FIG 44 18 TROUGH 16



Hebeloma ; Lactarius ; Paxillus

FIG 4419 TROUGH 17

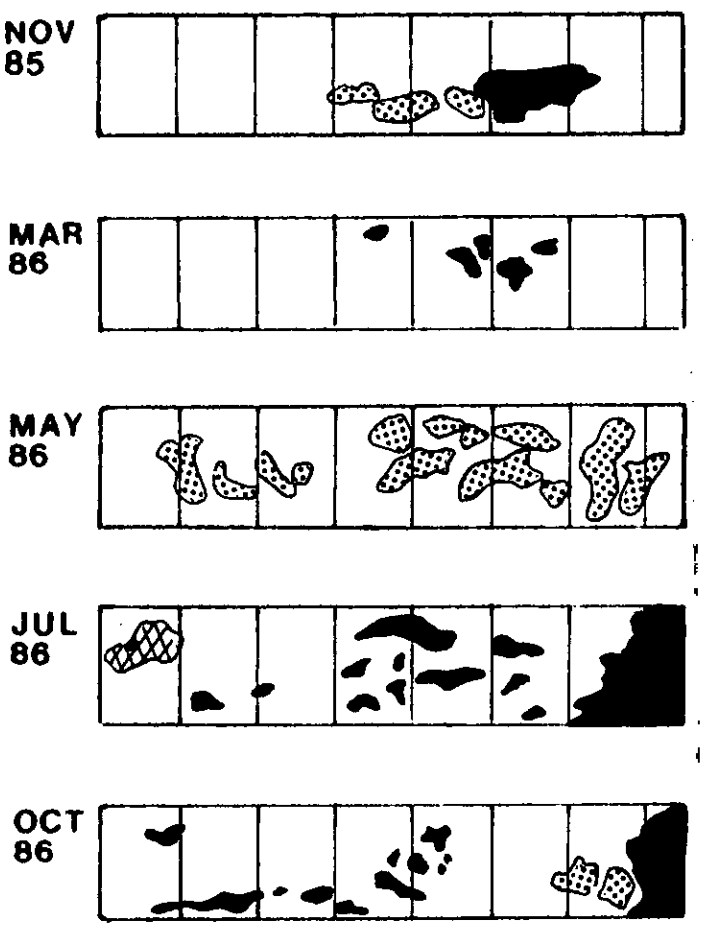
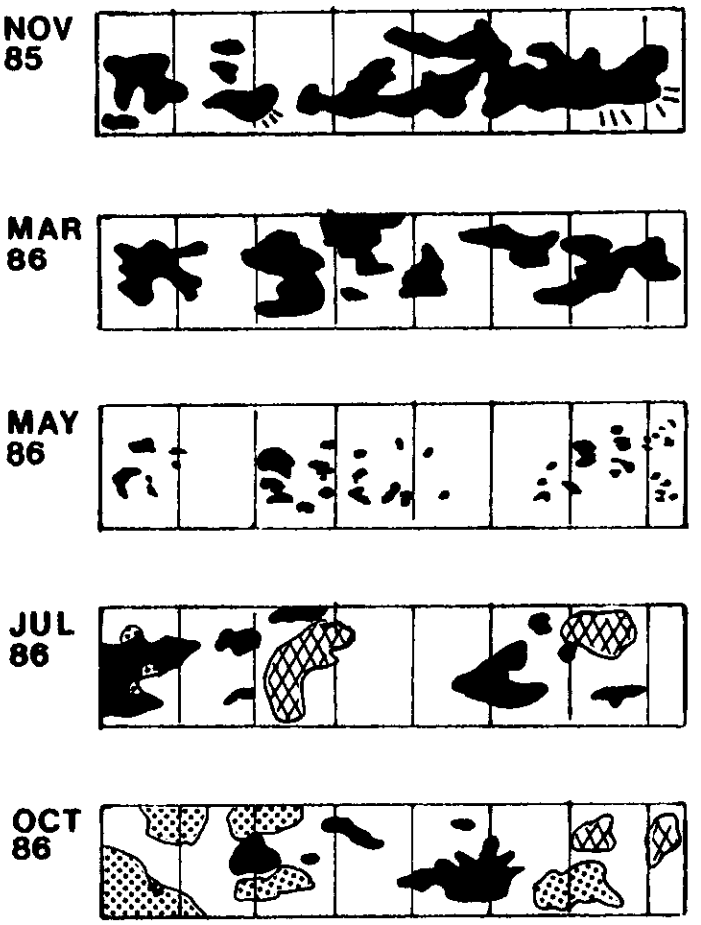


FIG 4420 TROUGH 18



Hebeloma ; Lactarius ; Paxillus

FIG 44 21 TROUGH 19

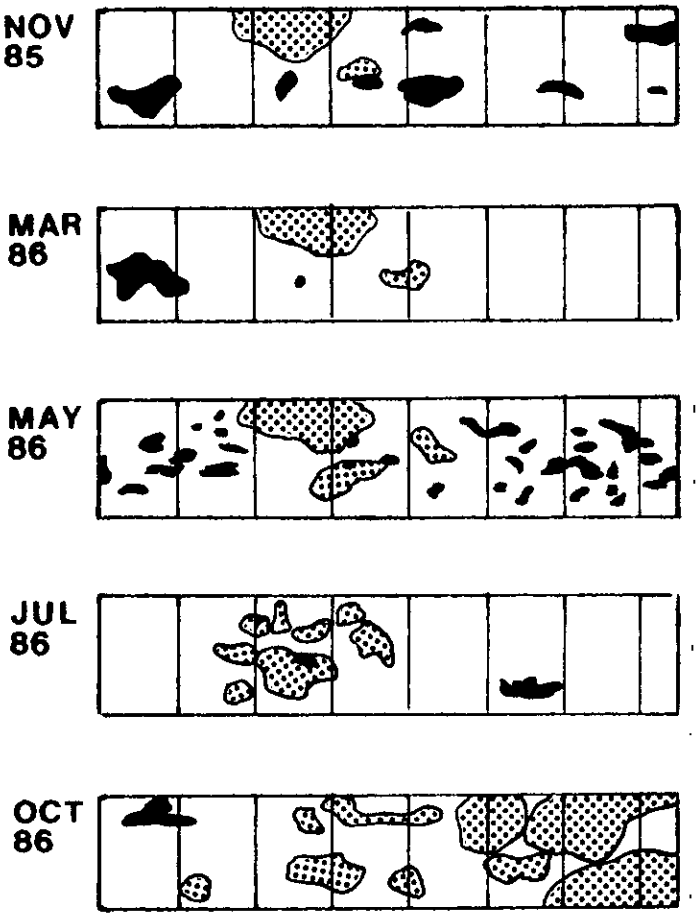


FIG 44 22 TROUGH 20

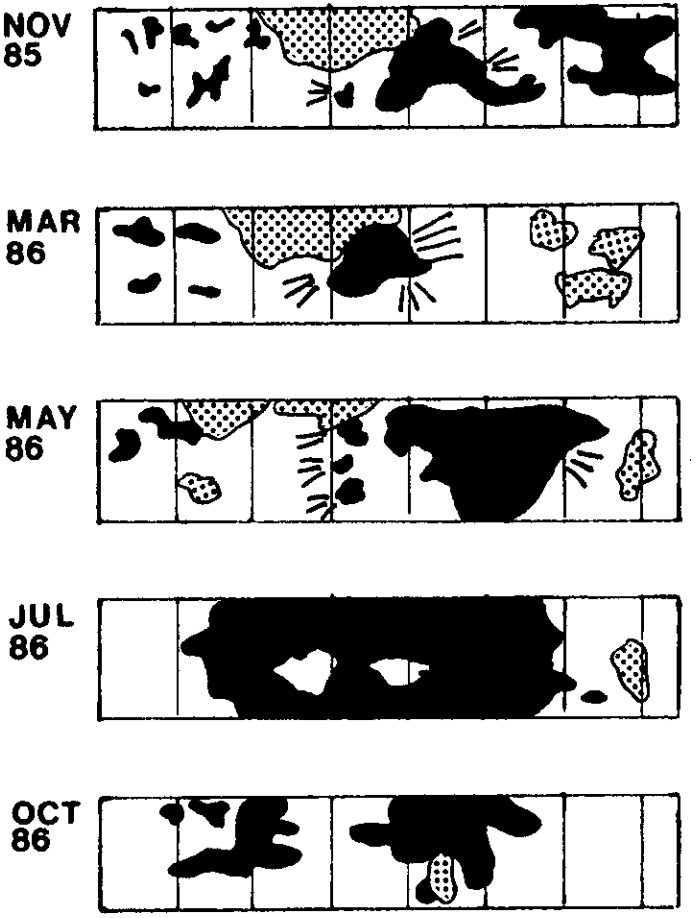


FIG 4 4 23 TROUGH 21

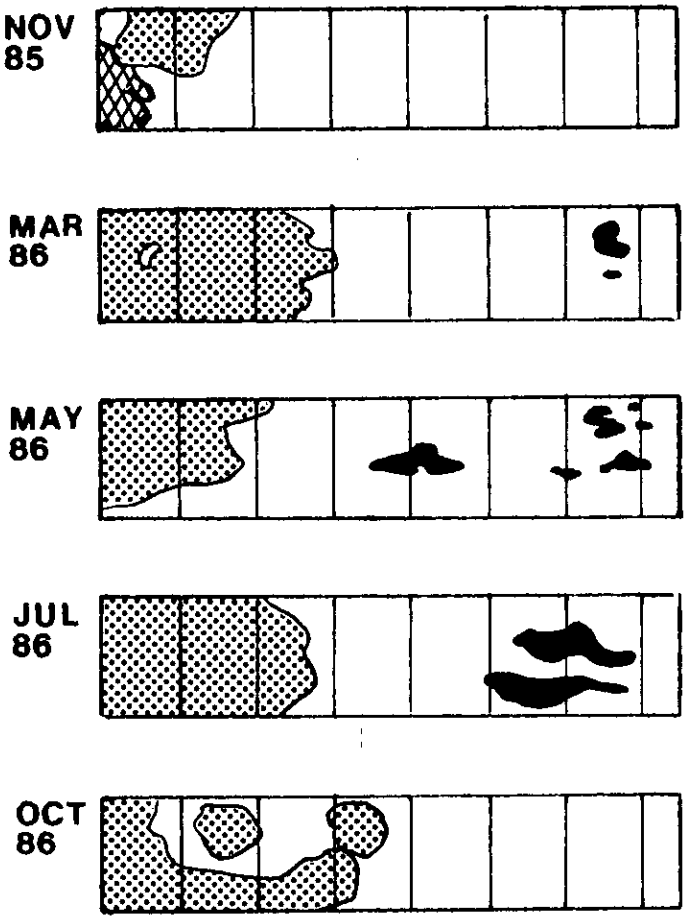
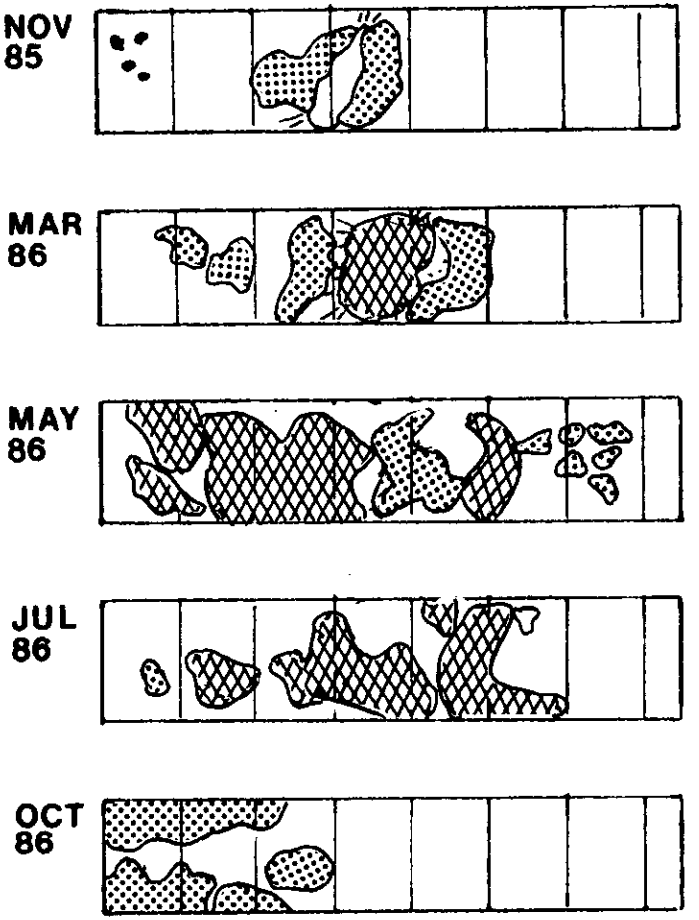


FIG 4 4 24 TROUGH 22



Hebeloma ; Lactarius ; Leccinum

FIG 4425 TROUGH 23

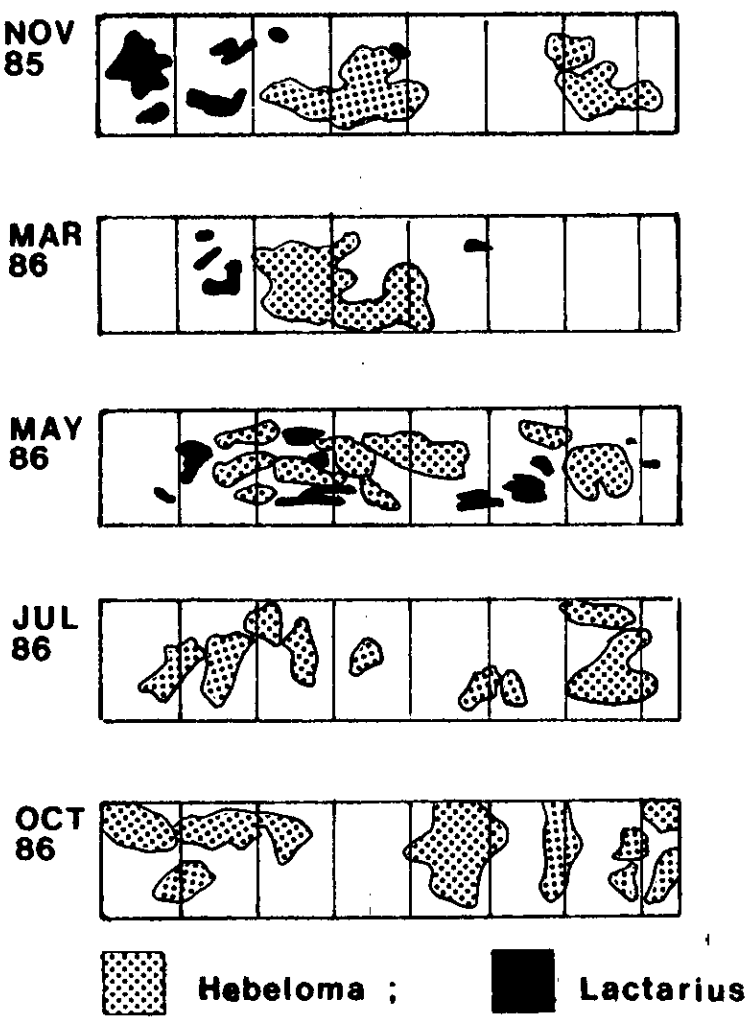


FIG 4426 TROUGH 24

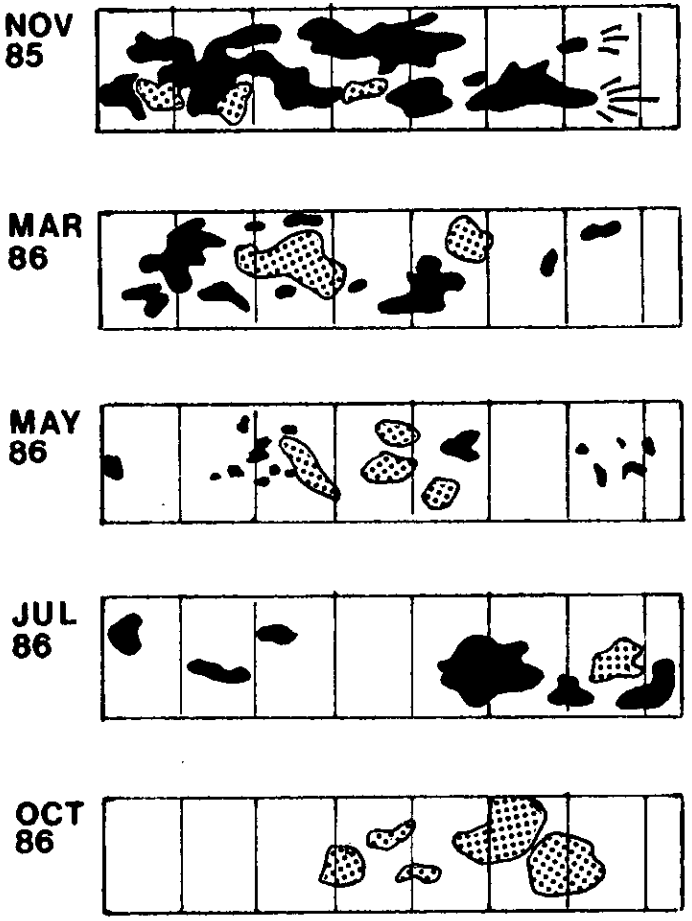


FIG 4427 TROUGH 25

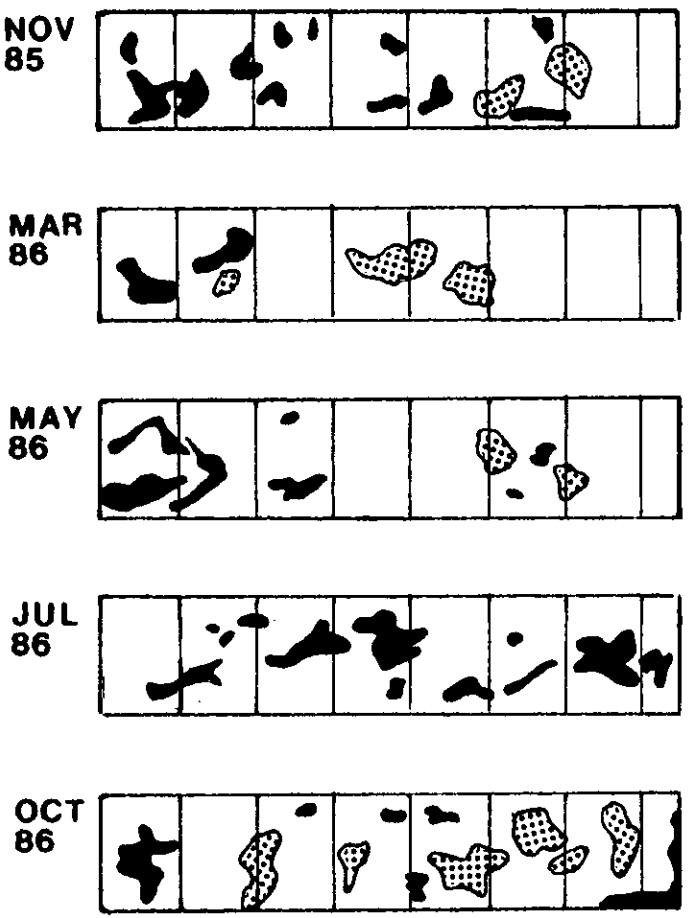
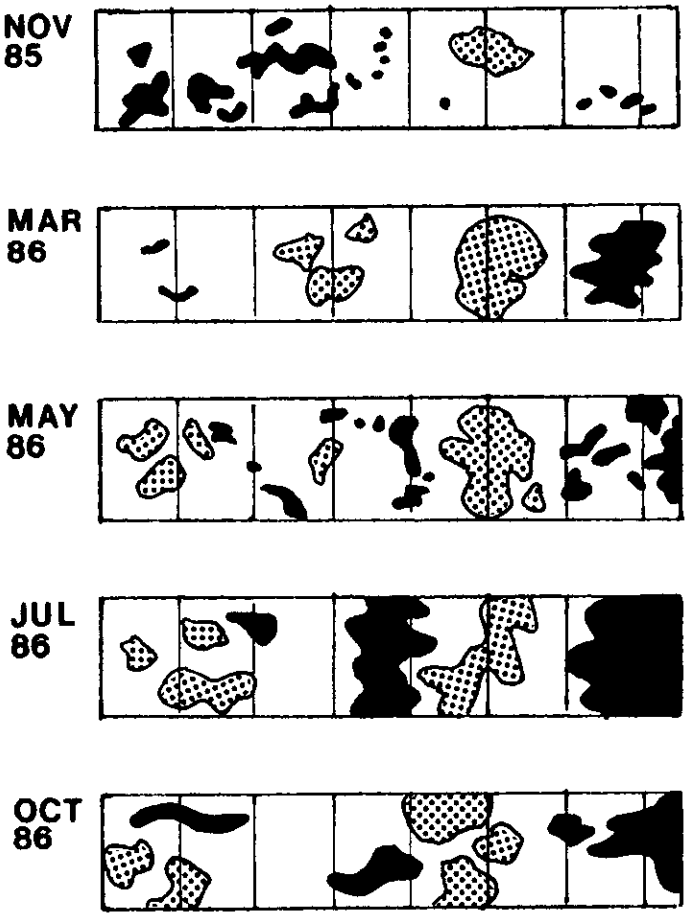


FIG 4428 TROUGH 26



Hebeloma ; Lactarius

FIG 4 4 29 TROUGH 27

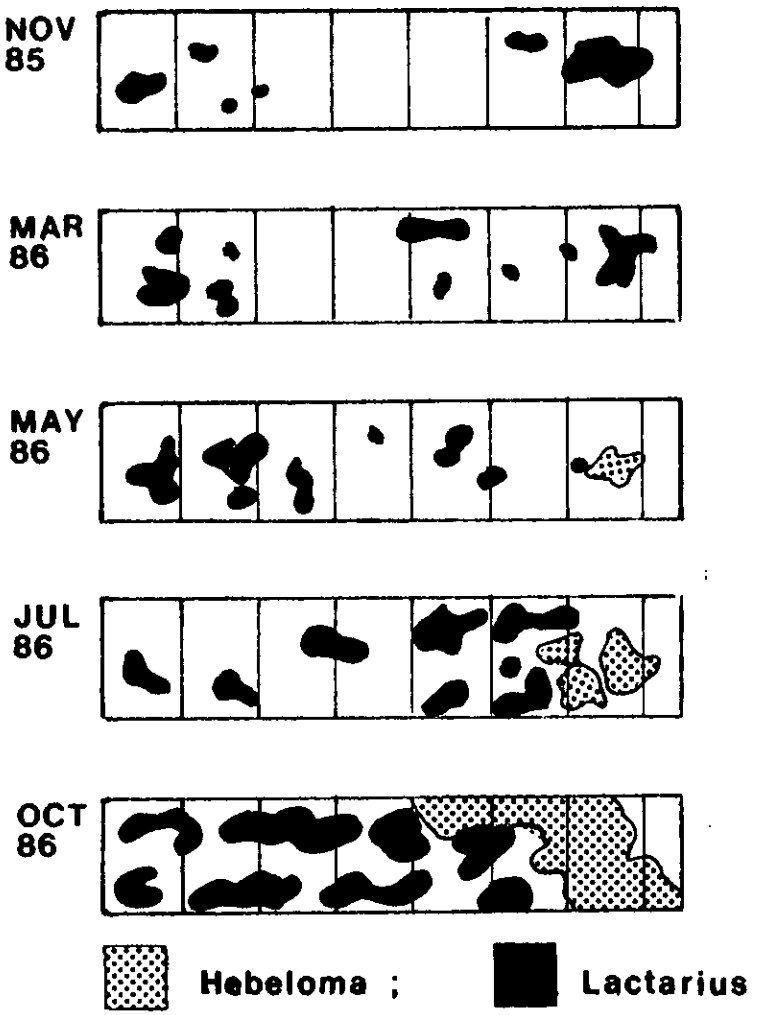


FIG 4 4 30 TROUGH 28

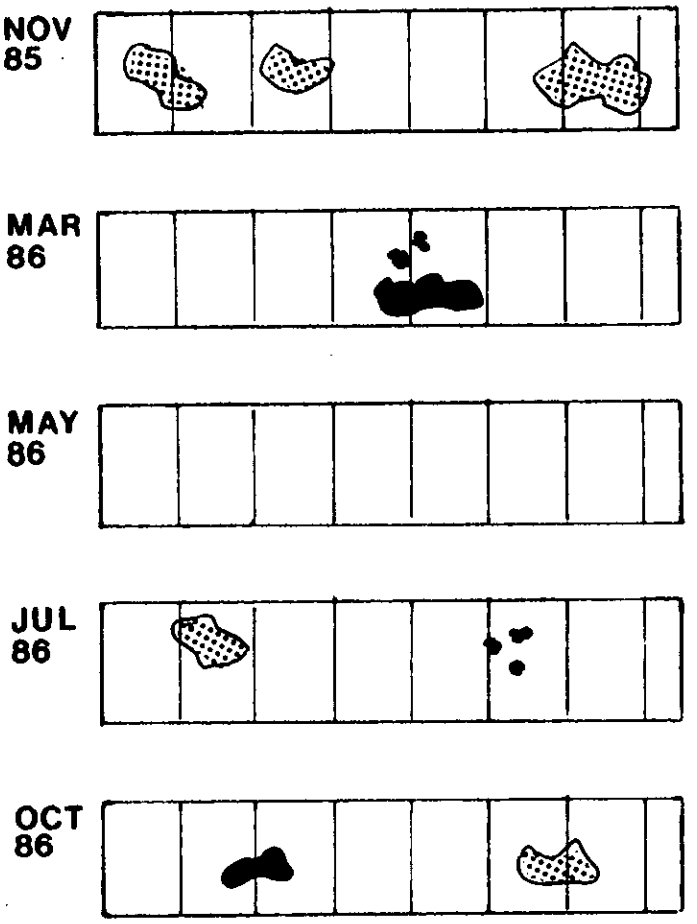


FIG 44 31 TROUGH 29

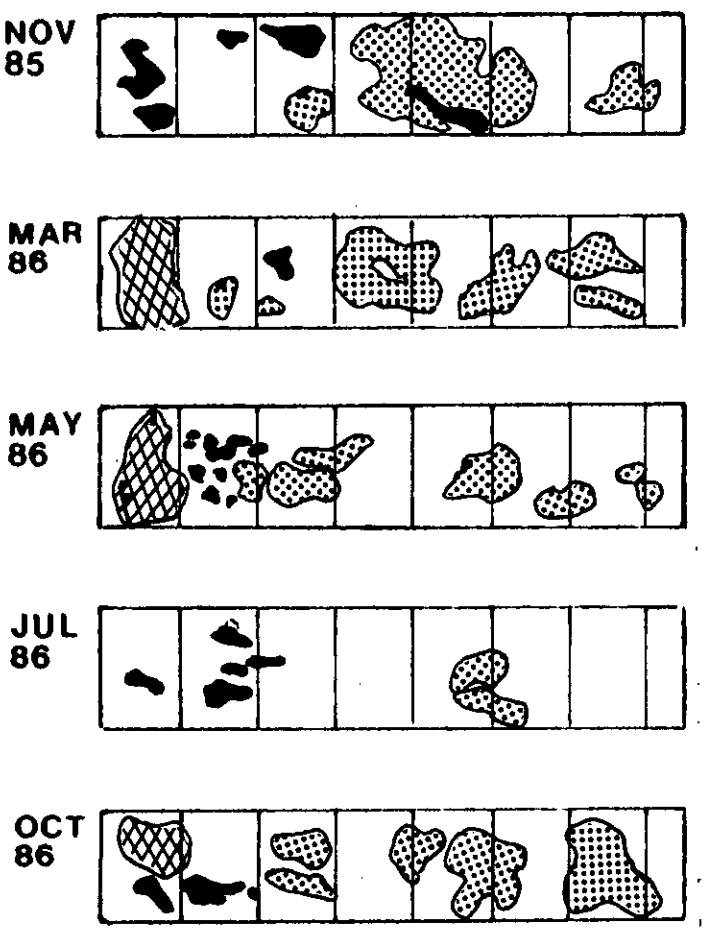
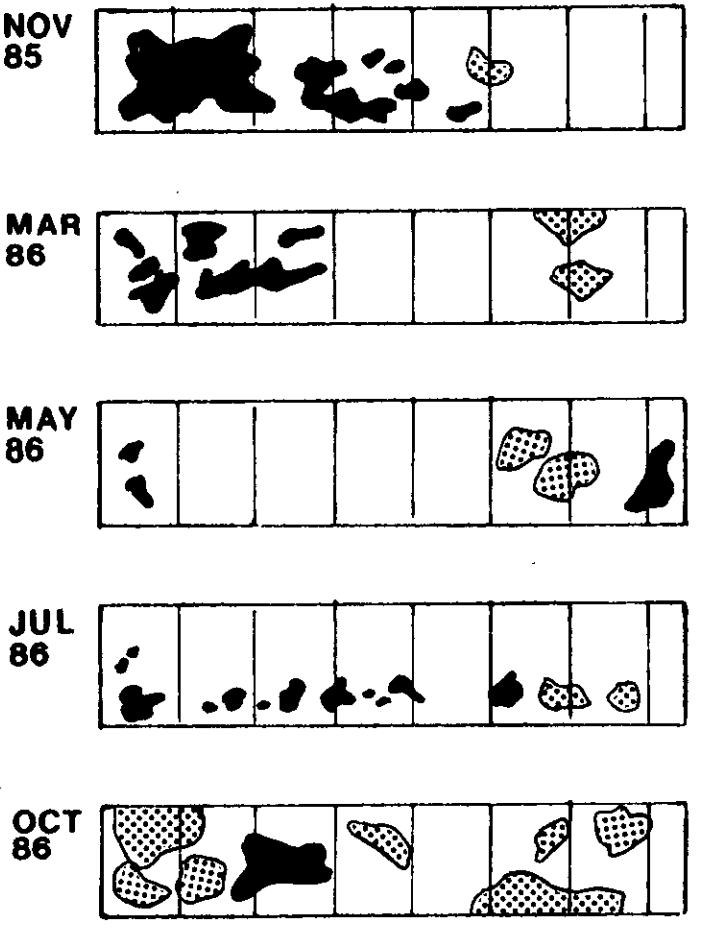


FIG 44 32 TROUGH 30



Hebeloma ; Lactarius ; Paxillus

FIG 4433 TROUGH 31

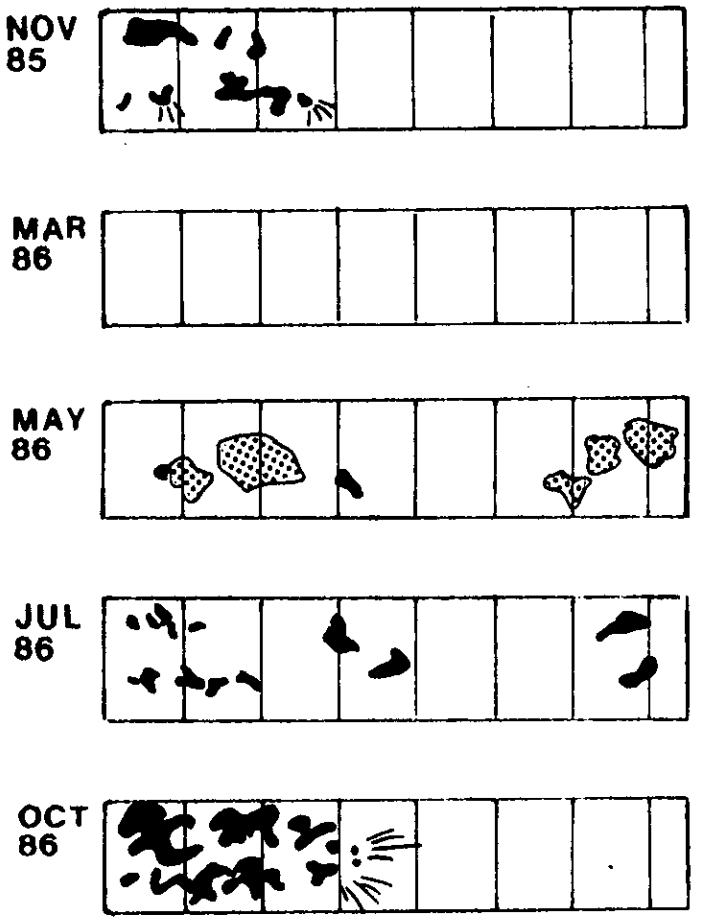
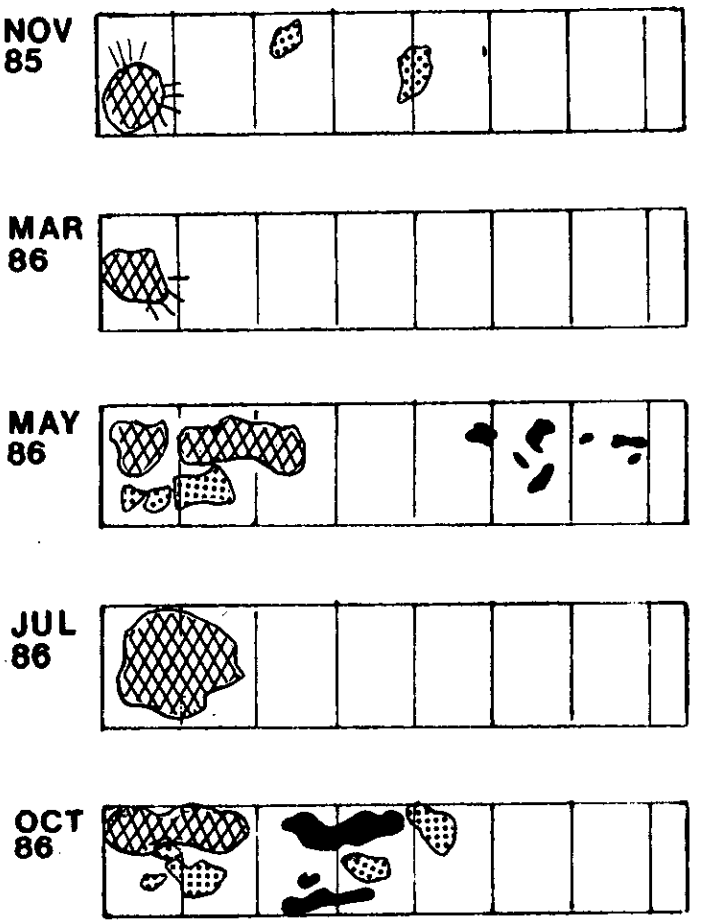


FIG 4434 TROUGH 32



Hebeloma ; Lactarius ; Leccinum

FIG 44 35 TROUGH 33

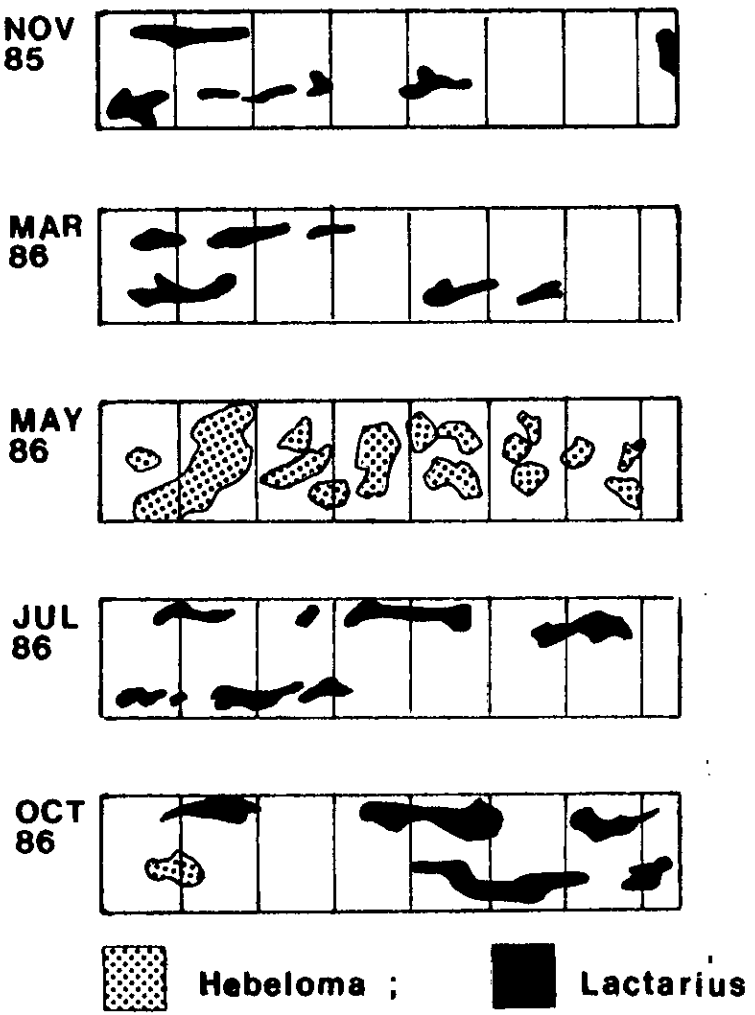


FIG 44 36 TROUGH 34

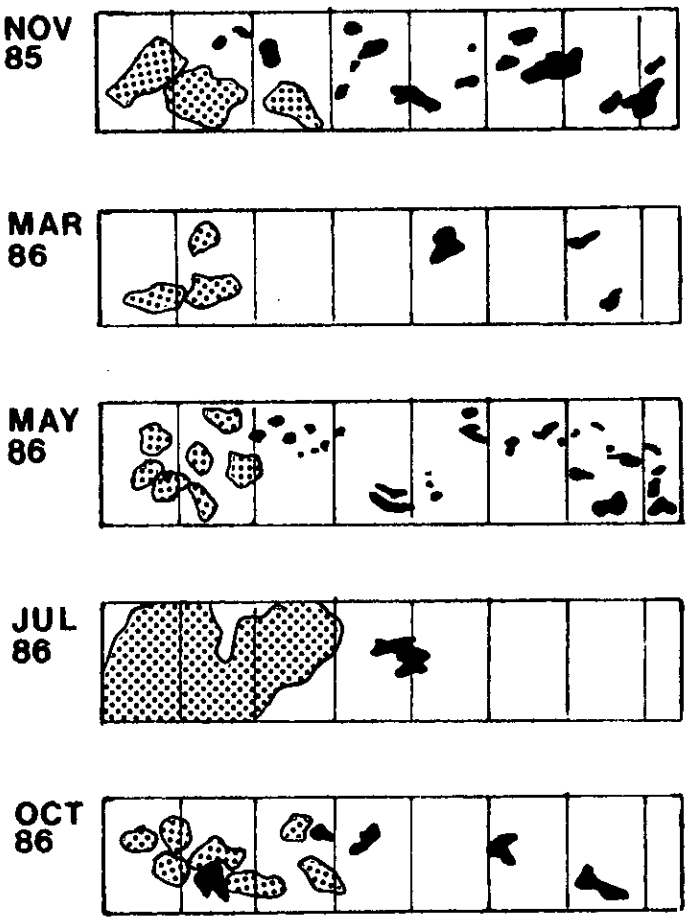


FIG 4437 TROUGH 35

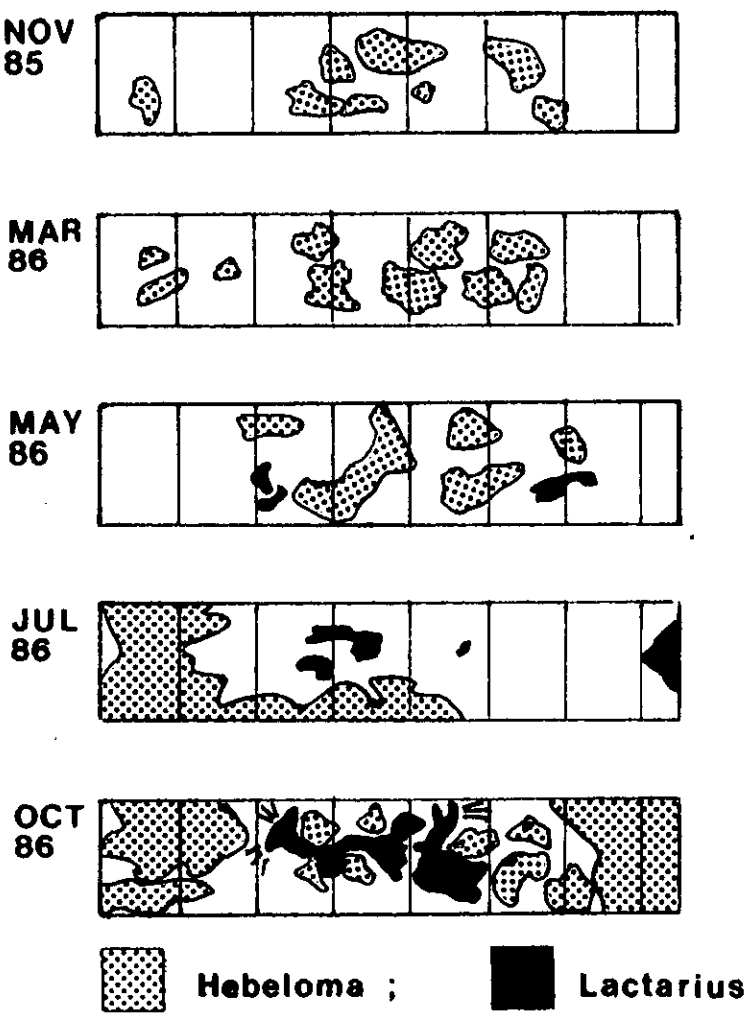


FIG 4438 TROUGH 36

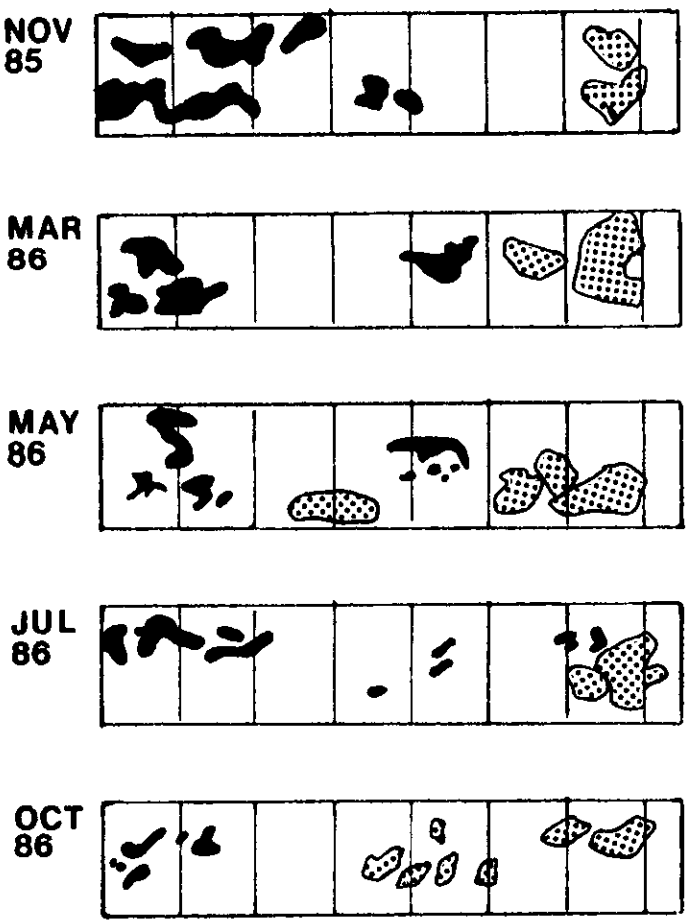


FIG 44 39 TROUGH 37

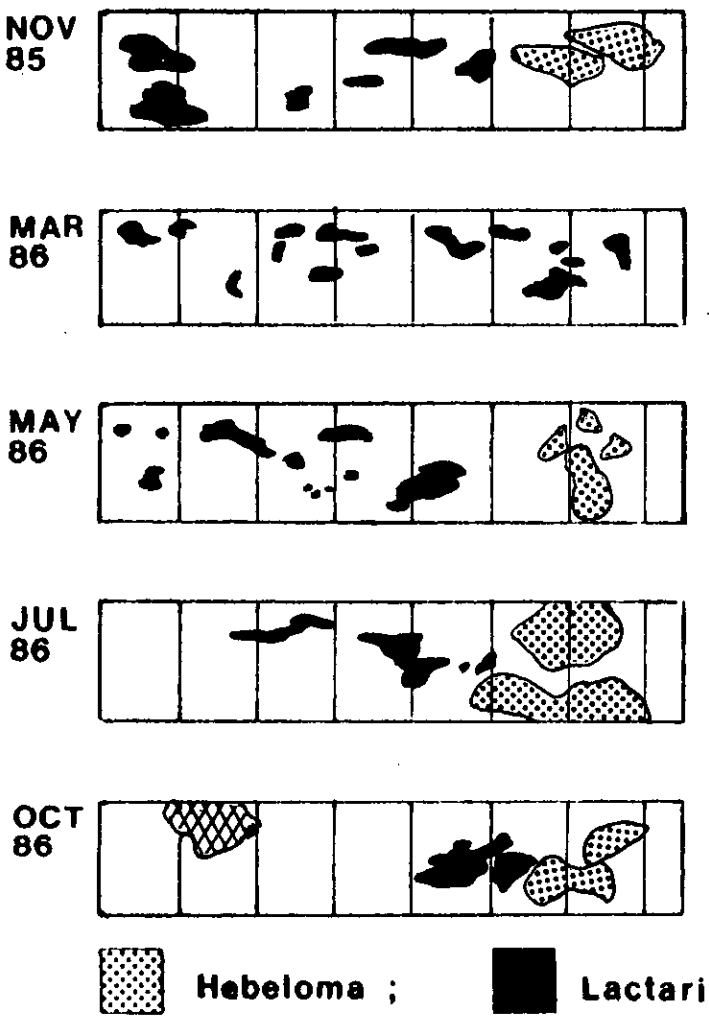
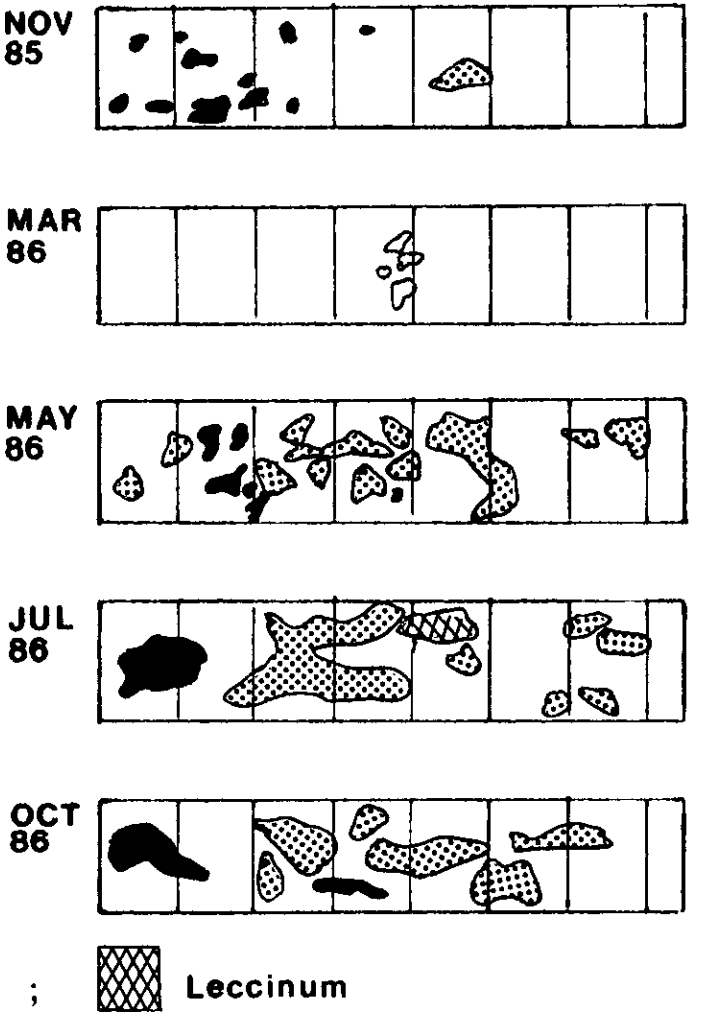


FIG 44 40 TROUGH 38



Hebeloma ; Lactarius ; Leccinum

FIG 4441 TROUGH 39

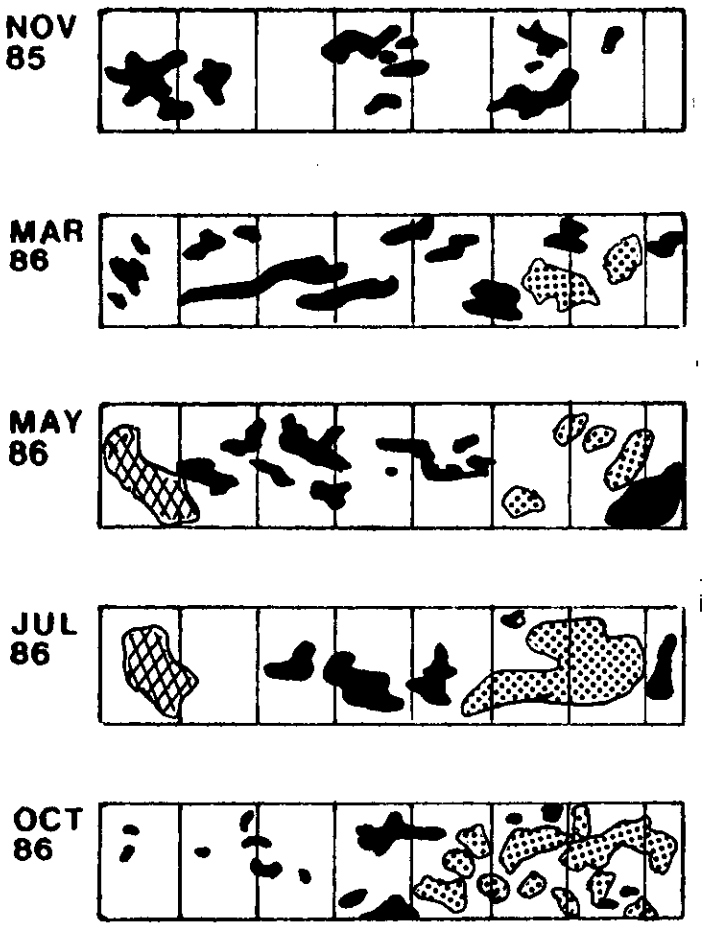
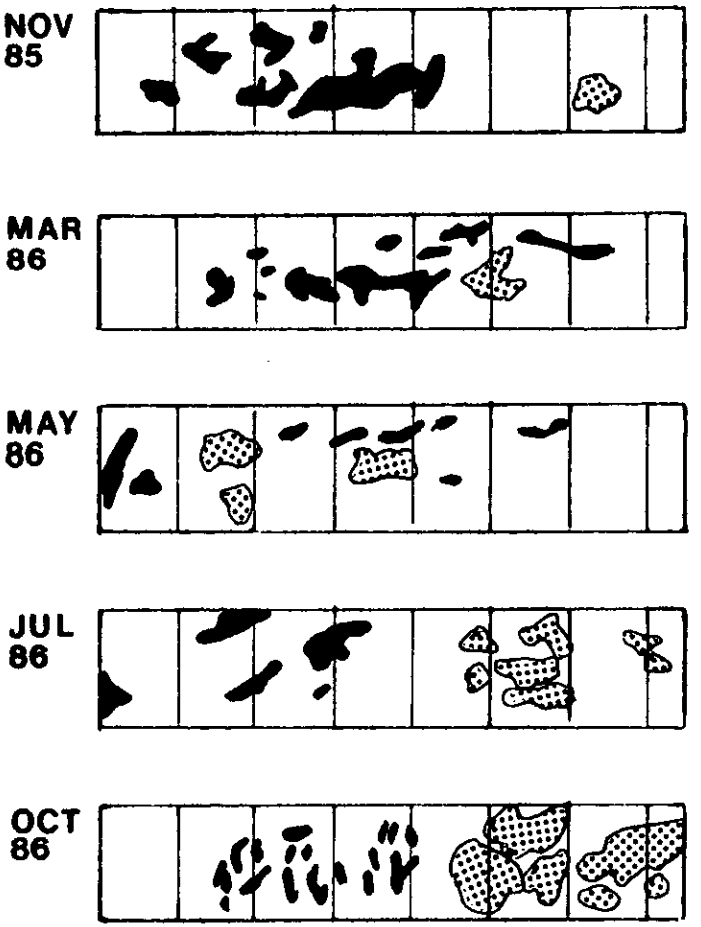


FIG 4442 TROUGH 40



Hebeloma ; Lactarius ; Paxillus

Lactarius-type mycorrhizas were associated with conspicuous mycelial strands as was often the case. In all instances, of course, only mycorrhizas that were present on the soil surface were recorded, and some that might have been very close to the surface were at times obscured by a thin film of slaked clay or silt that had been washed down the sides of the troughs. A final problem is that it was not feasible accurately to assess the density of mycorrhizal cover when mapping the distribution, so in some instances the records might relate to relatively few mycorrhizas of particularly conspicuous types that were present over a relatively large area, whereas in other instances localised distributions might have represented extremely dense populations. For all these reasons, it is appropriate first to consider the results of the final visual assessment (October 1986) which was carried out just before the troughs were destructively sampled. The results of this can be compared with those of microscopical counts - albeit in thin slices of soil, which did not correspond with the whole area assessed visually and which included mycorrhizas that were not visible on the soil surface. Such comparisons, as detailed below, can be used to assess the validity of the visual inspections.

Comparisons of visual and microscopical assessments

Hebeloma-type mycorrhizas were seen on the surface of 126 of the total 224 bands for the 32 troughs as a whole (Table 4.4.26), the bands being those from the centres of which thin slices of soil were taken for subsequent microscopical observations. For the 126 cases a mean 23 % cover by Hebeloma-type mycorrhizas was recorded, compared with 18 % of all root tips attributable to *Hebeloma* in bands containing Hebeloma-type mycorrhizas in the microscopical assessments of slices from the positions (calculated from the data in Table 4.4.13). For the 98 positions in which Hebeloma-type mycorrhizas were not seen on the soil surface, subsequent microscopical analysis of soil slices revealed a

Table 4.4.26
Percentage of the lower surface of troughs 9 - 40 that was covered by Hebeloma-type mycorrhizas in October 1986.

Tree	Sampling band						
	1	2	3	4	5	6	7
9	0	3	2	34	12	11	11
10	13	40	47	0	6	24	0
11	0	0	12	4	17	33	0
12	0	0	0	0	0	1	17
13	92	87	4	0	0	5	3
14	70	46	24	17	43	15	0
15	46	19	17	12	37	90	19
16	0	0	0	0	0	33	22
17	0	0	0	0	0	8	0
18	18	35	0	0	5	23	0
19	3	5	15	14	43	65	61
20	0	0	0	2	0	0	0
21	54	45	49	0	0	0	0
22	66	57	7	0	0	0	0
23	21	25	18	14	17	28	24
24	0	0	0	27	15	0	0
25	1	0	7	6	17	9	12
26	19	0	0	47	11	0	0
27	0	0	0	0	21	68	65
28	0	5	5	1	0	0	0
29	1	0	17	24	26	9	59
30	23	0	4	0	25	32	8
31	0	0	0	0	0	0	0
32	11	0	7	9	0	0	0
33	4	0	0	0	0	0	0
34	17	11	0	0	0	0	0
35	91	15	13	2	23	44	97
36	0	0	4	1	2	10	14
37	0	0	0	0	16	14	0
38	0	18	8	15	16	12	1
39	0	0	0	6	20	11	31
40	0	0	0	0	20	27	33

= sampling positions inoculated with *Hebeloma*

mean 6.6 % of root tips occupied by *Hebeloma*. Indeed, 39 of these positions had no Hebeloma-type mycorrhizas on microscopic examination, a further 37 had less that 10 % of root tips occupied by *Hebeloma* , only 22 had more than 10 % *Hebeloma*, and only 7 had more than 20 % *Hebeloma* (with, on average, 157 *Hebeloma* tips per sample - see Table 4.4.13). So visual observation failed to detect substantial development of Hebeloma-type mycorrhizas in only 7 positions in the whole 32 troughs (see Tables 4.4.13 and 4.4.26). Of the 126 positions in which Hebeloma-type mycorrhizas were seen by visual inspection, only 13 failed to reveal mycorrhizas of *Hebeloma* on microscopic examination of soil slices, and in these 13 cases the recorded surface cover by *Hebeloma* was low (mean 7 %). Overall, therefore, there was good agreement between the occurrence of mycorrhizas of *Hebeloma* recorded by visual inspection of the soil surface and subsequent microscopical assessments of root systems. This general agreement is illustrated in Fig 4.4.43 in which, for each trough, the percentage surface cover by Hebeloma mycorrhizas (totalled for all positions in which such mycorrhizas were observed) correlates with the percentage of Hebeloma mycorrhizas detected microscopically in soil slices taken from these positions (i.e. for 126 positions in all). In short, visual inspection of the soil surface in October 1986 satisfactorily reflected both the intensity of development and the distribution of Hebeloma mycorrhizas. On this basis visual assessments made at intervals during the course of the experiment (Figs 4.4.11 - 4.4.42) can be used with some confidence to detect changing patterns of development of Hebeloma-type mycorrhizas with time.

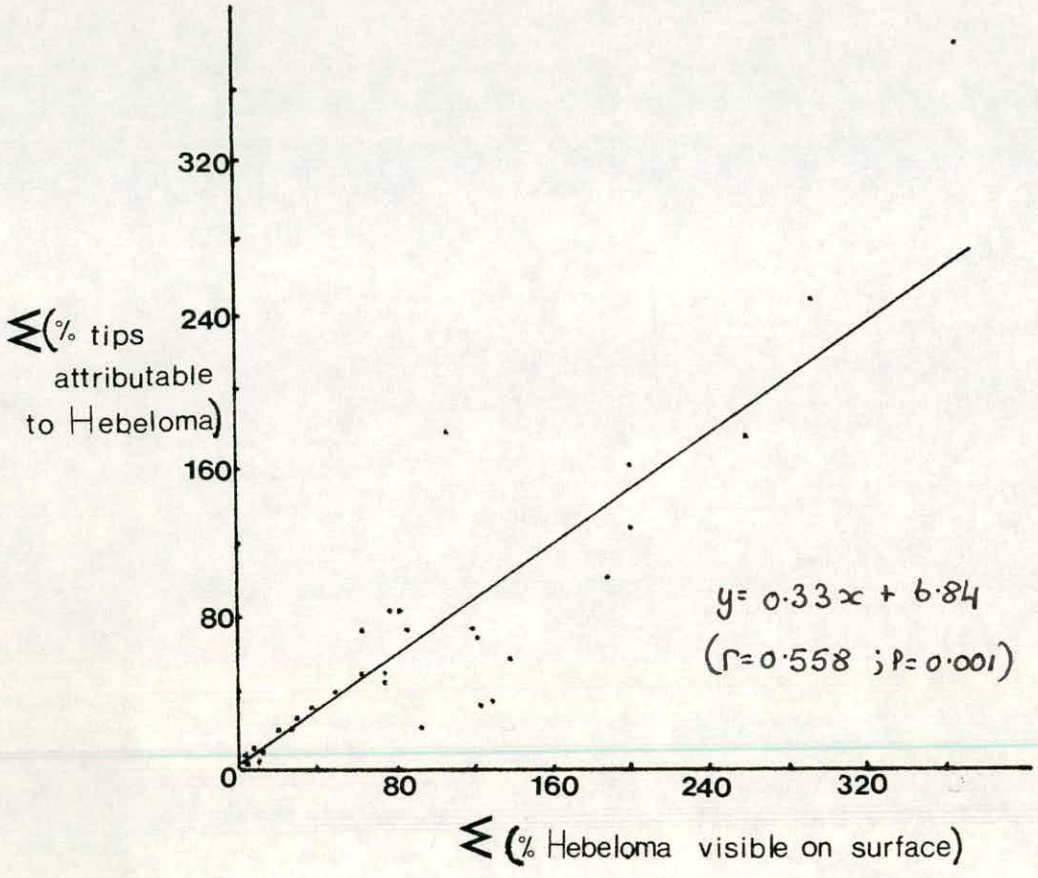
The situation regarding Lactarius-type mycorrhizas was more complex than for Hebeloma-type. By visual observation of the soil surface, Lactarius-type mycorrhizas were recorded in a total of 100 of the possible 224 bands in the 32 troughs (Table 4.4.27), compared with only 76 bands by microscopic observation.

Table 4.4.27
Percentage of the lower surface of troughs 9 - 40 covered by mycorrhizas of Lactarius-type in October 1896.

Tree	Sampling band						
	1	2	3	4	5	6	7
9	0	0	0	0	0	0	0
10	0	0	0	12	5	15	29
11	6	0	0	0	0	0	0
12	0	0	0	0	0	0	0
13	0	1	6	10	12	2	2
14	0	0	0	0	0	0	19
15	2	0	0	0	1	0	0
16	88	23	72	66	73	7	0
17	14	7	7	9	0	0	89
18	0	17	7	15	24	0	0
19	9	0	0	0	0	0	0
20	18	52	7	53	38	0	0
21	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0
25	9	15	1	4	1	0	11
26	11	1	17	4	0	9	55
27	26	52	45	40	13	0	0
28	1	5	6	5	7	7	9
29	15	6	0	0	0	0	0
30	0	21	9	0	0	0	0
31	26	23	0	0	0	0	0
32	0	14	11	0	0	0	0
33	15	0	6	26	9	1	15
34	7	0	5	0	6	2	2
35	0	25	29	37	1	0	0
36	4	1	0	0	0	0	0
37	0	0	0	0	0	0	0
38	25	0	5	5	0	0	0
39	1	2	2	4	4	1	1
40	0	6	9	6	2	0	0

= positions inoculated with *Lactarius*

Fig 4.4.43
Relationship between the percentage of the lower root surface with visible mycorrhizas of *Hebeloma* and the percentage of root tips attributable to *Hebeloma* in the equivalent "soil slices" as assessed by microscopy.



Also, the bands in which it was recorded visually and by microscopy did not always coincide (see Tables 4.4.14 and 4.4.27). Nevertheless, as in the case of *Hebeloma*, only a few (9) positions contained more than 10 % of root tips attributable to *Lactarius* when *Lactarius* was not detected visually and only 2 such positions contained more than 20% *Lactarius*. Conversely, in most instances (57 out of 100) in which *Lactarius* was recorded visually but not microscopically, the visual observations suggested less than 10 % cover of the soil surface. The predominance of Lactarius-type mycorrhizas in bands close to the tree base was detected visually as it was microscopically, with a mean of 99.3% surface cover in the three bands closest to the tree compared with a mean 51.9 % cover in the three bands furthest from the tree base. The difference, however, was not significant when compared by Student's t-test (t=1.6).

Leccinum was observed on the surface of only three troughs (32,22 and 37) in October 1986. In all three troughs *Leccinum* development was restricted to the two sampling bands closest to the tree base and accounted for only 5 % or 6 % of the surface cover on those bands.

Paxillus was observed on the surface of only one trough in October 1986 (trough 18). In this trough *Paxillus* accounted for 1 % and 5 % respectively of the surface cover in the two bands furthest from the tree base.

Patterns of development with time. Analyses presented in the previous section suggest that visual inspection of the soil surface in October 1986 satisfactorily reflected the distribution and the development of some of the main mycorrhizal types, and particularly of Hebeloma-type mycorrhizas. On this basis, the sequential visual assessments in Figs 4.4.11 - 4.4.42 can be used to determine general changes in mycorrhizal development in the troughs during the

course of the experiment.

Inspection of Figs 4.4.11 - 4.4.42 reveals considerable variation between troughs in a) the predominant types of mycorrhizas present, b) the positions of these mycorrhizas and c) changes in positions of mycorrhizal types with time. As an example of (a) above, trough 14 had a predominance of Hebeloma-type mycorrhizas whereas trough 20 had little *Hebeloma* but a predominance of *Lactarius*. To illustrate (b) above, trough 16 had Hebeloma-type mycorrhizas apparently confined to regions furthest from the tree, whereas Hebeloma-type mycorrhizas were generalised or tended to predominate closest to the tree in trough 14. Lastly, an example of (c) above is provided by comparison of trough 16, in which the mycorrhizal types tend to remain in the same positions during the experiment, and trough 22 in which there is fairly clear evidence of a change in the distribution of Hebeloma mycorrhizas with time. In order to detect major trends, therefore, the troughs must be grouped according to various criteria. Troughs 12, 28 and 33 will be excluded from further analysis because they contained few visible mycorrhizas, with no obvious pattern of occurrence. For the remaining 29 troughs, 22 showed a generally consistent pattern over the five assessment times (representing the end of one growing season and four periods during the second season) and only 7 showed a substantial change of pattern. The troughs concerned are as follows:

1) Pattern of occurrence of different mycorrhizal types
remains more or less consistent over the five assessment times:
troughs - 9, 11, 13, 14, 16, 19, 20, 21, 23, 25, 26, 27, 29,
30, 31, 34, 36, 37, 38, 39, 40.

2) Pattern of occurrence of different mycorrhizal types changes substantially over the course of the five assessment times:

troughs - 10, 15, 17, 18, 22, 24, 35.

The second group of troughs mentioned above showed various patterns of change including, for example, a progressive restriction of Hebeloma-type mycorrhizas to regions closest to the tree base (trough 22), a progressive shift in the occurrence of Hebeloma-type mycorrhizas towards the ends of the trough coupled with substantial development of Lactarius-type mycorrhizas in the middle region (trough 35), and a progressive decrease in incidence of Lactarius-type mycorrhizas near the ends of the trough coupled with development of Hebeloma-type mycorrhizas in the end regions (trough 18). The majority of troughs, however, as listed above, showed a high degree of uniformity between sampling times, as best exemplified by troughs 9, 14, 16, 21, and 34. On this basis, there was little evidence of spatial or temporal successional patterns of mycorrhizal development in the troughs, as are reported around trees in field conditions.

The 29 troughs (excluding 12,28 and 33) can be grouped according to other criteria - for example, whether there is a general trend towards increase or decrease in incidence of different mycorrhizal types with time. On this basis, 20 troughs (10, 11, 13, 14, 15, 18, 19, 21, 23, 24, 25, 26, 27, 30, 32, 35, 37, 38, 39 and 40) showed a general increase in incidence of Hebeloma-type mycorrhizas with time of incubation. Six troughs (9, 16, 22, 29, 34, 36) showed no major change in *Hebeloma*, one trough (20) showed a decrease in *Hebeloma*, and two troughs (17 and 31) could not be analysed because the incidence of *Hebeloma* was too low or erratic.

Similar analysis to that above but for Lactarius-type mycorrhizas reveals that 8 troughs (10, 13, 16, 17, 27, 31, 32 and 36) showed a decrease in *Lactarius*, and 12 troughs (11, 14, 20, 21, 22, 25, 26, 29, 37, 38, 39, 40) showed no

major change or could not be analysed.

Of interest, there were several cases in which Lactarius-type mycorrhizas were clearly seen in November 1985, accompanied by mycelial strands (eg. in troughs 18,20,24,26, and 31), so in these instances this mycorrhizal type was present at the ends of the troughs furthest from the tree bases, even at the end of the first growing season when it would have been present on root regions newly formed in that season at the periphery of the root system.

Mycorrhizas of *Leccinum* (in 4 troughs) and *Paxillus* (in 3 troughs) tended to remain in constant positions at successive assessment times, usually near the tree base or in the middle regions of the troughs. They did not show clear evidence of increase or decrease with time, although in four troughs these types did not persist on the surface until the final recording, and in trough 22 Leccinum-type mycorrhizas evidently decreased in incidence towards the end of the experiment.

4.5. Discussion.

The two experiments reported in this section involved the development of methods for studying spatial and temporal aspects of mycorrhizal successions on root systems in closely controlled environmental conditions. It is appropriate to consider first the degree to which the experimental design proved useful in these respects and then to consider the major findings from these experiments.

Throughout the 3 years during which the troughs were maintained in a glasshouse, they were found to be suitable for periodic examination of the tree root systems with minimal disturbance to the soil or mycorrhizas on the bottom face of the soil. Indeed, the polythene overlays that were used during periodic inspection of the root systems enabled a permanent record to be kept of the main positions of development of different mycorrhizal types, so that changes in abundance or position of these types could be monitored. The only significant limitation in this respect was that only mycorrhizal types visible and distinguishable with the naked eye were recorded, although small samples of the main mycorrhizal types could be removed for more detailed microscopical examination and identification. Comparisons of the recorded distribution of mycorrhizal types immediately before harvest with the results of detailed microscopical examination of mycorrhizas in "soil slices" taken at the end of the experiments revealed very close agreement in the case of Hebeloma-type mycorrhizas. So, much confidence can be placed in the analysis of changes of distribution of these mycorrhizas with time of incubation of experiment. Unfortunately, the comparisons proved less satisfactory for Lactarius-type mycorrhizas, suggesting that these had been misidentified in some instances by visual inspection alone.

The advantage of using naturally occurring saplings from a coal spoil heap was apparent early in the course of these experiments. The plant shoot systems were small, having been grazed repeatedly by rabbits, so they did not cause the loss of much water by evapotranspiration in the early stages of the experiments - a feature of considerable advantage in view of the relatively small total volume of soil present in each trough. The root systems, however, were large and could be aranged longitudinally in the troughs so that differently aged root regions could be inoculated with selected mycorrhizal fungi. Moreover, the root systems were easily freed from adhering coal spoil when collected from the original field site, and they bore only a restricted range of mycorrhizal fungi, mainly *Paxillus* and *Scleroderma* spp., typical of coal spoil sites. These mycorrhizal types were known to decline in abundance on transfer of the plants to brown earth as used in the troughs(Fleming, 1983 a) and this was confirmed by observations during the experiments.

The design of the experiments enabled the positions of occurrence of fungal fruitbodies to be recorded and compared with the relative abundance of mycorrhizas in equivalent positions in the troughs , this being achieved by non-destructive sampling of the root systems. So the experiments enable some comparisons to be made with the more extensive data available on distribution of fruitbodies around trees in field sites (Ford *et al*, 1980;Mason *et al*, 1982; Last *et al*. 1984 a,b).

Inoculation of different parts of the root systems also was facilitated by the design of these experiments, although such inoculations gave variable results. In the first experiment, there was little or no evidence of establishment of mycorrhizas of either *Hebeloma sacchariolens* or *Lactarius pubescens* when vermiculite-peat inocula of these fungi were applied uniformly along the lengths

of the troughs (Tables 4.3.4 and 4.3.5). The reasons for this failure were not investigated, but it is possible that too little inoculum was applied or that it was applied wrongly. In particular, the inoculum was distributed in only a narrow strip along the extreme base of each trough, where it would have been subject to periodic saturation with water draining into the base of each trough when these were watered. No mycorrhizas of *Lactarius* spp. were observed in either of the first two sampling positions of the troughs in this experiment, whereas Lactarius would have been expected to develop from the inoculum at these sampling positions. Moreover, only a total of twelve sclerotium-like bodies typical of *H.sacchariolens* (Fox,1986) were seen on the root systems in the first experiment as a whole. Many Hebeloma-type mycorrhizas were observed but the absence of sclerotium-like bodies indicates that these mycorrhizas had not developed from the added inoculum but had developed from other, naturally occurring, inoculum of *Hebeloma*. The inoculation procedure was modified for the second experiment. Liquid culture inocula were used and were applied to the whole of the under surface of the soil within defined bands. Also, the inoculations were repeated after 14 weeks. Inoculum of *H.crustuliniforme* was used instead of *H.sacchariolens*, and both Hebeloma- and Lactarius-type mycorrhizas then developed in response to inoculation by *H.crustuliniforme* and *L.pubescens* respectively (Table 4.4.12). In contrast, there was no evidence that mycorrhizas of *Laccaria* developed in response to inoculation with *Laccaria proxima*. But the isolate of *L.proxima* used in this experiment was subsequently found to be only weakly infective in other glasshouse experiments, perhaps because it had lost its infectivity during prolonged storage.

A final advantage of the experimental design developed in this work was the fact that young, aseptically grown seedlings could be placed at intervals along the troughs and subsequently retrieved to compare their mycorrhizal status

with that of adjacent parts of the root systems of the older trees. However, major problems were encountered in retrieving the root systems of seedlings inserted into the centres of the troughs, because the root systems had intermingled with those of the older trees, and such seedlings always grew very poorly. These problems were subsequently overcome by inserting seedlings down the sides of the troughs, where they grew well and their root systems could be recovered more or less intact.

In the first experiment reported here, each trough contained two plants, one at each end, and their root systems were arranged so that they would overlap as they grew. In this way, it was hoped to compare mycorrhizal development on, respectively, young and old parts of root systems occupying the same region of soil in each trough, so that any possible localised influences of soil conditions could be excluded. Unfortunately, this design proved impractical. At an early sampling time, two troughs were destructively sampled and an attempt was made to separate the overlapping root systems after prolonged soaking of the soil cores in water. Even with extreme care, many of the fine roots and fascicles of mycorrhizal tips were lost during the attempted separation and it was felt that few meaningful results could be obtained. At a subsequent sampling it was decided to dissect out only a few of the main longitudinally running roots from each trough, selecting those that were most readily accessible at the soil surface when the troughs were inverted. Again, however, many of the mycorrhizal tips were lost in this process. At the final sampling of this first experiment, therefore, the original objective was largely abandoned, especially as it had become clear that there was no significant mycorrhizal development from added inocula. Instead, the troughs were sampled by taking slices of soil at intervals along the troughs, and no attempt was made to distinguish between the roots of the two trees in each trough. This sampling

method was easy to standardise and involved no selection of roots that may or may not have been typical of the root systems as a whole; moreover, it enabled all of the mycorrhizal tips to be retrieved. This sampling procedure was used throughout the second experiment, in which only one tree was incubated in each trough.

Sequential observations of mycorrhizas visible on the soil surface at the bases of the troughs showed, in general, a large degree of stability of mycorrhizal development. In other words, the main regions of occurrence of different mycorrhizal types did not differ greatly over the course of the experiments. In some instances, however, it was noticed that mycorrhizas of *Lactarius* (with associated mycelial strands) developed initially near the periphery of the root system but tended to be replaced by other mycorrhizal types, or were not visible on the soil surface, later in the course of the experiments. Mycorrhizas of *Leccinum* and, to a lesser degree, *Paxillus* sometimes occurred in abundance early in the experiments but their incidence decreased with time until, at the final sampling, they were not visible or else occupied only a small area of the soil surface. The interesting feature, overall, was that there was little conclusive evidence of successional patterns in the troughs, and certainly no strong evidence equivalent to that obtained by observations of mycorrhizal distribution or distributions of fruitbodies in field plots. Nevertheless, some of the observations from these experiments may relate to successional patterns, as described below.

In general, mycorrhizas of *Lactarius*, *Leccinum* and *Cenococcum* predominated on the older parts of the root systems, and this was clear from both microscopical assessments and visual observations of the lower surface, although sequential recordings did show that Lactarius-type could develop, but

not neccessarily persist, at the periphery of the root system too. Other fungi, such as *Hebeloma* spp., *T.terrestris* and *P.involutus*, occurred more or less uniformly along the roots (Experiment 2, Sample 2) and mycorrhizas of *Inocybe* spp., although present all along the root systems, tended to become increasingly dense with increasing distance from the tree base. In all of these respects it must be remembered that observations could only be made over a relatively short time span (maximum 2 years); it would be desirable to have longer periods of study, corresponding to the sequential observations in field sites, in order to investigate a larger proportion of the successions reported from field studies. The trees in this set of experiments reached a maximum age of 5 years and from the fruitbody mapping studies at I.T.E., it seems that such trees would be old enough to support only the early- and, perhaps, some late-stage fungi in the succession (Last *et al*,1983). The only true "late-stage" species observed in these experiments was *Leccinum*, because *Lactarius* has been shown to have characteristics of both "early-" and "late-stage" groups and perhaps would be best placed in a "middle-stage" group (Fleming *et al*,1984). Perhaps if it were prolonged for several more years, this type of investigation could yield information to corroborate or contradict the successional patterns derived from fruitbody mapping studies.

The variation in the results between the troughs also suggests that the mycorrhizal development in early years may depend largely on chance establishment of various early-stage types, and perhaps only with time would the influence of such chance events diminish and reveal a clearer picture of successional events mediated by host or environmental factors.

Fleming *et al.* (1984) found that seedlings planted initially in "mycorrhizal" or "non-mycorrhizal" soils developed mycorrhizas of *Hebeloma* and

Inocybe respectively and these types persisted on the roots of the seedlings for 4 years. The seedlings that became mycorrhizal with *Inocybe* spp. remained predominantly infected with this type for the duration of the experiment but those initially infected with *Hebeloma* spp. gradually became infected with *Lactarius* and *Inocybe* in the later years of the experiment. Fleming *et al.* (1985) also showed in other experiments that Lactarius-type mycorrhizas, arising from naturally occurring inoculum, tended to develop on seedlings previously infected by *Hebeloma* but not those infected by *Laccaria*. Thus the fungi that infect the root tips of young seedlings can apparently influence the ability of other species to establish mycorrhizas in later years of a tree's growth.

Inocybe-type mycorrhizas were present , in varying numbers, on almost every root system examined in these two experiments and this perhaps is a reflection of the persistence of this mycorrhizal type, in contrast to others that were initially present, on the roots of saplings taken from the coal spoil. However, initial observations of root systems from the coal spoil site revealed no evidence of conspicuous Inocybe-type mycorrhizas. In the first sample of Experiment 1, there was evidence that *Inocybe*, unlike *Hebeloma* or *Lactarius*, was unable to infect the overlapping root system of another sapling growing in the same trough as a tree bearing Inocybe-type mycorrhizas. Tree 3, in sample 1 of Experiment 1, bore mycorrhizas of *Inocybe* but Tree 4, in the same trough, was infected only with *Hebeloma*. Unfortunately, owing to the sampling problems discussed earlier, the assessments did not involve quantification of mycorrhizal development, so it is possible that *Hebeloma* infected tree 4 to such an extent that root tips were not available for colonisation by *Inocybe*; it is also possible that tree 4 was genetically unsuitable to infection by *Inocybe* (see Section 5.4).

The use of seedlings planted into the troughs proved useful in

corroborating these findings concerning the apparent inability of *Inocybe* to "cross-infect". Non-mycorrhizal seedlings planted into, or down the sides of, troughs in Experiment 2 became mycorrhizal predominantly with Thelephora-type (34% of seedling root tips overall) followed, in order of decreasing quantity, by Hebeloma-type, Inocybe-type and Lactarius-type, with some instances of Laccaria, Cenococcum, Paxillus and Leccinum types. Whereas the seedlings that developed mycorrhizas of Hebeloma-type and Lactarius-type were in positions where the older tree root system also had high levels of these respective types, the degree of development of Inocybe -type on seedling root systems was independent of the presence (or amount) of Inocybe-type on root systems of the older trees in the same regions of the troughs. Thus, *Inocybe* again was seen to have poor ability to infect roots of one plant from mycorrhizas that were present on adjacent roots of another plant. In Experiment 2 sample 2, however, *Hebeloma* and *Lactarius* seemed readily to infect seedling roots from established mycorrhizas on the roots of older plants. These observations suggest the need for further experiments, and they may relate to the relative paucity of mycelial strands or other hyphal aggregates on mycorrhizas formed by *Inocybe* spp., whereas such aggregates are common on mycorrhizas of *Lactarius* (Fleming,1983 a) and have been suggested to function in spread of mycorrhizal development to seedlings planted around mature trees (Fleming, 1983 a,b)

The seedlings on Experiment 1 that were originally infected (>50% of tips) with either Hebeloma-type or Thelephora-type ultimately had much lower proportions of their root systems infected by these fungi (means of 7.8% and 21.5% of tips attributable to *Hebeloma* and *Thelephora*, respectively) in October 1986. Moreover the roots became mycorrhizal with *Inocybe*, *Thelephora* or *Lactarius*, irrespective of the original mycorrhizal types present on them. The mycorrhizal status of the older tree root system beside which the seedlings were

positioned had no apparent effect on the mycorrhizal types that developed in the seedling roots; seedlings placed beside regions of the older root system with dense establishment of Hebeloma or Lactarius-type did not develop mycorrhizas of these types. There was evidence, however, that the presence of *Lactarius* on the older tree roots resulted in significantly greater infection of seedling roots with *Inocybe* than did the presence of *Hebeloma* on the older tree roots. The reason for the unusual behaviour of *Inocybe* is again unclear and merits further investigation. The replacement of *Hebeloma* or *Thelephora* on the seedling roots in the course of the experiment, although significant, was not complete even on such small root systems - but the smalln ess of the recovered root systems precluded attempts to identify parts of them in which either *Hebeloma* or *Thelephora* had been replaced. Thus, although the root systems were small and the time scale short, there was evidence of progressive replacement of mycorrhizal types in glasshouse conditions, but original infections by "early-stage" species were not entirely eliminated.

Fruitbodies of mycorrhizal fungi were observed in almost all troughs, both in Experiment 1 (7 out of 8 troughs) and Experiment 2 (29 out of 32 troughs), and the types and numbers of fruitbodies and their positions along the troughs reveal several interesting points.

Whereas it was proposed by Deacon *et al.* (1983) that mycorrhizas of particular fungi were most likely to be found below or near fruitbodies of those species, it was not clear from my results that this was necessarily the case. There was no significant difference in the percentage of mycorrhizas attributable to *Thelephora*, *Inocybe* or *Hebeloma* - the main types of mycorrhizal fungi that fruited - in positions beneath fruitbodies of the appropriate type compared with positions where no fruitbodies were observed. Indeed, in Experiment 2 there were

significantly more fruitbodies of *Inocybe* in sampling positons 1-3 (close to the tree) than in positions 5-7 (furthest from the tree base) but the proportion of mycorrhizal tips attributable to *Inocybe* increased significantly with distance from the tree base. At least in the conditions of my experiments, therefore, it seems that the development of fruitbodies was determined by factors unrelated to the mycorrhizal status of the roots.

The times of the appearance of fruitbodies in the course of the experiments are also worthy of mention. In both experiments, before trees went into dormancy in response to natural changes in the environment, the fruiting "season" lasted for 19,7 and 13 wk for troughs in Experiment 1 in 1984,1985 and 1986 respectively and 13 wk for troughs in Experiment 2, in both 1985 and 1986. In all cases there was evidence of an early (June) flush of *Inocybe* fruitbodies, and in Experiment 2 this fungus fruited again in August. It also appeared that these two flushes came earlier in 1986 than in 1985 (see Tables 4.4.21 and 4.4.22) but this may have reflected the weather conditions in these two summers, as the two separate flushes of *Thelephora* fruitbodies in Experiment 1 in 1985 occurred later that did the single flush of *Thelephora* in 1986.

In the second experiment three types of mycorrhizal fungi fruited in 1985 but five types (excluding *Peziza* spp.) fruited in 1986. This follows the pattern of temporal succession reported from the studies at I.T.E.,Bush, wherein the numbers of mycorrhizal types fruiting around the trees in the experimental plot increased with time. Also it is notable that one of the two types that fruited in the second, but not the first, year was *Lactarius*, regarded as a middle - late stage mycorrhizal species. It is perhaps coincidental that the only occurrence of a Lactarius-type fruitbody was in a trough inoculated at each sampling position with liquid inoculum of *L.pubescens* and also inoculated with spores of

L.pubescens directly below the position of fruiting. It is true that there was no effect of the application of spores of *L.pubescens* in any of the other 15 positions to which they were applied, but this was the only occasion in which spores, liquid inoculum and close proximity to the tree base all coincided. It would be interesting to study further the efficacy of combinations of inoculum types and density of application along the root systems in relation to fruitbody development by *Lactarius*.

From the first appearances of fruitbodies in Experiment 1, when saplings were subjected to artificial heating and lighting to encourage bud formation prior to imposed dormancy, it seems that the mycorrhizal populations on the root systems of the saplings, after only 4 months' growth, were sufficient to produce a flush of fruitbodies, and that fruiting is governed by the physioligical status of the host plant.

Thus the study of fruitbody production in the troughs in these two experiments followed , in general, the temporal successional pattern observed in field conditions. Indeed, fruitbodies of both *Hebeloma* spp. and *Lactarius* spp. first appeared relatively close to the tree bases.

In summary of this section, the experimental method developed in these experiments seems to provide a workable method of studying in detail the changes in mycorrhizal status of root systems of individual plants with respect to time and distance from the tree base. The experiments also provided a basis for comparison with the fruitbody and microscopical studies carried out in field plots at I.T.E.,Bush Estate over the last 16 years. Although my results corroborate the observations of those previous studies in several respects, notably in predominance of some mycorrhizal types close to the bases of trees, they also highlight areas of difference - for example in the density of mycorrhizas below

fruitbodies, and these areas, in particular, merit further investigation.

CHAPTER 5

MISCELLANEOUS ASPECTS OF THE PHYSIOLOGY AND INFECTIVITY OF MYCORRHIZAL FUNGI.

A number of different and largely preliminary studies were made during the course of this research, essentially to seek evidence of differences in the behaviour of proposed early- and late-stage fungi in the mycorrhizal successions on birch. Some of the results of these studies are presented in this chapter.

5.1. Effects of different concentrations of glucose, nitrogen and phosphorus on growth and mycorrhizal establishment by representative mycorrhizal fungi in axenic conditions.

5.1.1. Introduction

Although a few fungi which can form mycorrhizas can exist as saprophytes (Harley, 1966) most are ecologically obligate biotrophs (Lewis, 1973) in the sense of Garrett (1970), depending on the roots with which they form mycorrhizas for their source of carbon (Lewis,1975). Melin & Nilsson (1957) showed that plants supply carbohydrates to mycorrhizal fungi, and Lewis & Harley (1965 c) indicated that this movement is mainly unidirectional, the carbohydrate being converted in the fungal sheath to the "fungal carbohydrates" mannitol and trehalose and the storage polysaccharide glycogen - sources that the plant cannot use. An early proposal that mycorrhizal development would occur only on roots containing high levels of soluble sugars was made by Björkman (1942). This proposal seemed worthy of reinvestigation insofar as early

and late stage mycorrhizal fungi might differ in carbohydrate demand because they seem characteristically to be associated with trees of different age. The possible difference in carbohydrate demand of early and late stage mycorrhizal fungi prompted two of the experiments described here.

In a related context, as trees age and accumulate mineral nutrients such as nitrogen and phosphorus there is an inevitable depletion of these nutrients from the soil and this,too, might be a determinant of the different infective abilities of early and late-stage mycorrhizal fungi.Therefore experiments also were performed to investigate this possibility.

5.1.2. General method

Modified Melin-Norkrans (MMN) agar was prepared with variations to achieve reductions from "standard" in concentration of glucose, phosphorus or nitrogen (see Section 2.1.2).

In one experiment the agar was sterilised by autoclaving at 121°C for 15 min and then added, in aseptic conditions, to sterile transparent polypropylene specimen tubes (9 cm tall, 3 cm diameter) with screw top lids, each with a central hole plugged with cotton wool. The tubes, each with 15 ml agar, were sloped at 45°C during cooling of the agar . In the other two experiments the agars were autoclaved in 17 ml amounts in boiling tubes plugged with cotton wool bungs and capped with aluminium foil. Again, the tubes were sloped during cooling.

All agar slopes were inoculated with single discs, 6 mm diameter, cut from the growing margins of fungal colonies on plates of MMN agar. The tubes were then incubated at 25°C for 14 days, by which time the fungi had started to grow from their inocula. Control tubes received a disc of sterile MMN agar, and

were incubated as for the inoculated tubes.

Aseptically germinated seedlings of *B.pubescens*, approximately 4 weeks old, were placed on the agar slopes, one per tube, such that the roots were in contact with the inoculum discs. The tubes were then incubated at an angle of 45° beneath banks of fluorescent "Gro-lux" tubes supplying continuous light in a laboratory at 23°C. The experimental design was of randomised blocks in which one tube of each treatment, plus controls, comprised a block. After 12 weeks' incubation, the experiments were sampled and the seedlings were assessed for mycorrhizal development, root dry weight and shoot dry weight.

During the course of these experiments, agar plates were prepared with the appropriate concentrations of nutrients as used in the tubes. They were inoculated centrally with 6 mm diameter inoculum discs of the fungi and incubated in darkness at 23°C. Colony radii were measured at intervals, over several weeks if necessary, along two orthogonal lines marked on the bases of the plates. From these recordings the mean radial growth rates on three replicate plates of each type of agar were calculated.

5.1.3. Effect of glucose concentration on fungal growth and mycorrhizal development.

In this experiment, MMN agar was prepared containing glucose at concentrations of 0,0.01, 0.1 or 1 %. Specimen tubes containing these agars were inoculated with *Laccaria proxima* isolate 19, *Hebeloma crustuliniforme* isolate 4, *H.sacchariolens* isolate 4, *Paxillus involutus* isolate 16, *Lactarius pubescens* isolate 4 or *Amanita muscaria* isolate 5 and subsequently planted with sterile birch seedlings. After 12 weeks' incubation, the seedlings were removed from the tubes and assessed for shoot height and shoot dry weight,the number of root

tips and the proportion of these that had become mycorrhizal (Tables 5.1.1 - 5.1.4). Radial growth rates of the six fungal isolates also were determined on plates of MMN agar containing different concentrations of glucose (Fig 5.1.1).

Seedling shoot heights (Table 5.1.1) and dry weights (Table 5.1.2) were markedly influenced by glucose concentration. Overall, the best shoot growth occurred in the presence of 0.01 and 0.1 % glucose, slightly less growth occurred in the absence of glucose, and growth was extremely poor in the presence of 1.0% glucose. These findings were largely unaffected by the type of fungus used for inoculation, except that plants grew particularly poorly in the absence of glucose when inoculated with either *P.involutus* or *A.muscaria*. Unfortunately, no non-inoculated plants were included in this experiment. In contrast to the effects of glucose, the type of inoculant fungus did not, overall, significantly influence plant growth.

Contrary to its effects on shoot growth, the presence or concentration of glucose did not significantly affect the number of root tips on the seedlings (Table 5.1.3). In particular, the presence of 1% glucose did not have nearly such a marked effect on the number of root tips as it did on shoot growth parameters. The different mycorrhizal fungi did, however, significantly affect the number of root tips, with particularly high numbers being formed in the presence of *L.pubescens* at all glucose levels.

A generally high proportion of root tips became mycorrhizal with all of the test fungi (Table 5.1.4), but there were major differences between glucose treatments in this respect and evidence also of interaction between fungi and glucose treatments in the degree of mycorrhizal development. *L.proxima* developed mycorrhizas on a similar proportion of root tips at all glucose levels, as did *H.sacchariolens*. The other four fungi, however, infected a much larger

Tables 5.1.1 - 5.1.4 Effect of glucose concentration in MMN agar on the growth and mycorrhizal development of birch seedlings incubated for 12 wk and inoculated with different mycorrhizal fungi.(Means of 10 replicates).

Table 5.1.1
Shoot length (mm)

Fungus	Glucose content 0%	0.01%	0.1%	1%	Row Mean		
Lpx19	8.4	9.5	13.1	0.4	7.8		
Hc4	9.0	10.6	12.6	0.4	8.1		
Hs4	8.2	11.8	11.0	0.6	7.9		
Pi6	4.0	11.5	11.3	0.4	6.8	SED	1.6
Lp4	11.4	10.4	11.3	1.1	8.5	5%LSD	-
Am5	2.6	8.3	12.7	3.2	6.7		
Column Means	7.3	10.3	12.0	1.0			
		SED	5.63				
		5%LSD	5.63				

Table 5.1.2
Shoot dry weight (mg)

Fungus	Glucose content 0%	0.01%	0.1%	1%	Row Mean		
Lpx19	10.8	7.9	8.5	0.7	7.0		
Hc4	7.3	6.1	10.4	0.5	6.1		
Hs4	3.9	8.5	5.2	0.7	4.6		
Pi6	1.8	6.4	6.7	0.4	3.8	SED	2.5
Lp4	9.6	11.5	0.7	0.8	5.6	5%LSD	-
Am5	0.9	5.0	8.8	1.8	4.1		
Column Means	5.7	7.6	6.7	0.8			
		SED	2.31				
		5%LSD	4.77				

Table 5.1.3
Mean number of root tips per seedling

Fungus	Glucose content				Row Mean		
	0%	0.01%	0.1%	1%			
Lpx19	41	24	32	15	28.0		
Hc4	22	21	32	9	21.0		
Hs4	11	21	15	15	15.5		
Pi6	11	25	24	15	18.7	SED	6.8
Lp4	33	39	31	32	33.7	5%LSD	14.0
Am5	3	18	29	19	17.2		
Column Means	20.2	24.7	27.2	17.5			
		SED	4.84				
		5%LSD	-				

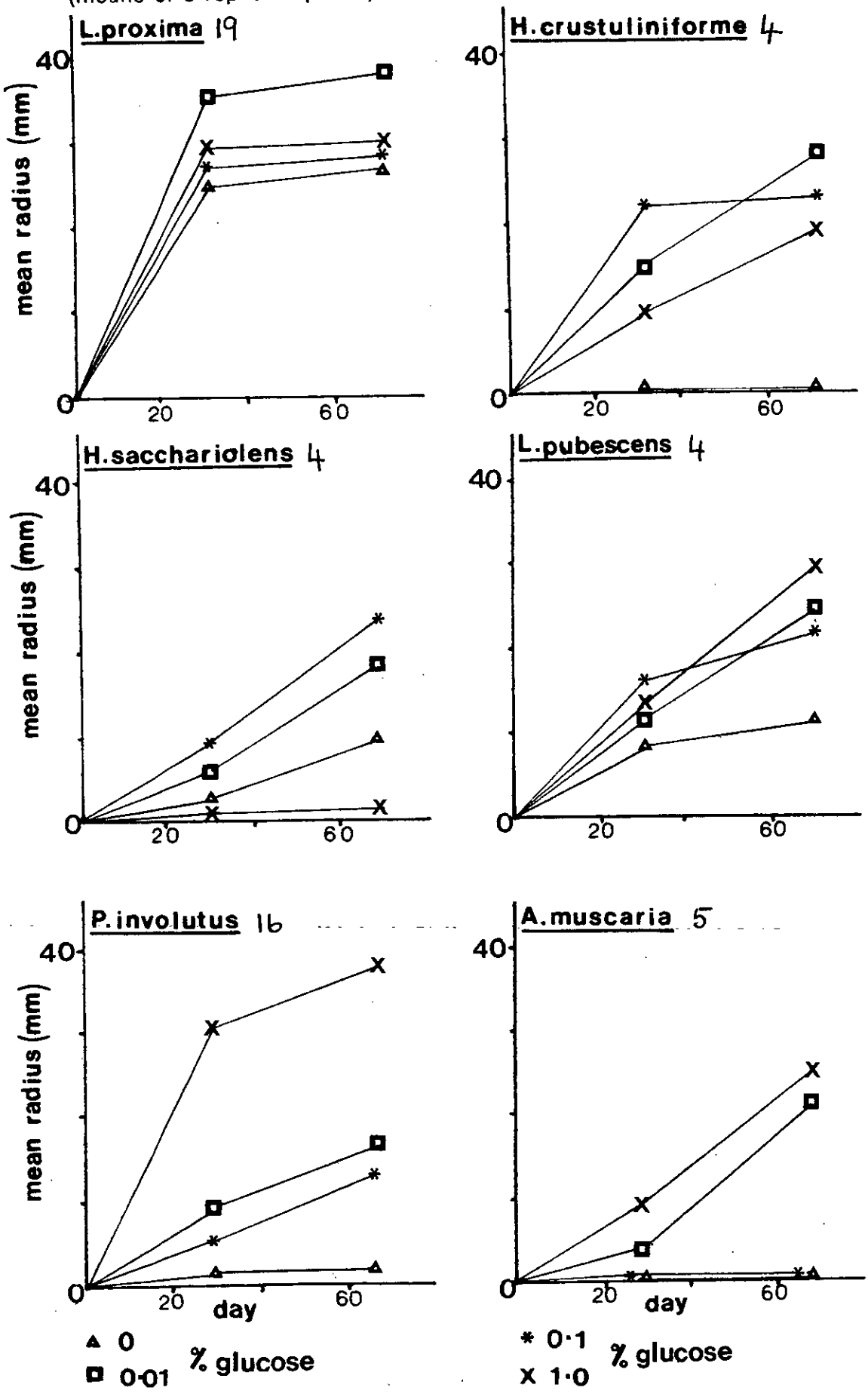
Table 5.1.4.
Mean percentage of mycorrhizal tips per seedling

Fungus	Glucose content				Row Mean		
	0%	0.01%	0.1%	1%			
Lpx19	56	58	55	56	56.2		
Hc4	47	55	45	96	60.7		
Hs4	67	34	53	62	54.0		
Pi6	75	60	46	87	67.0	SED	12.6
Lp4	10	29	21	82	35.5	%5LSD	26.0
Am5	31	34	31	64	40.0		
Column Means	47.7	45.0	41.8	74.5			
		SED	4.73				
		5%LSD	9.80				
		1%LSD	13.23				
		0.1%LSD	17.71				

proportion of root tips in the presence of 1% glucose than in all other treatments, such that in the experiment as a whole the proportion of mycorrhizal tips at the highest glucose level was significantly (P=0.001) greater than at all other glucose levels. There was no simple explanation of this phenomenon; comparison of Tables 5.1.3 and 5.1.4, for example, shows no evidence that it was simply related to the number of root tips present on the seedlings.

On agar plates, all of the fungi made poor linear growth in the absence of glucose (Fig 5.1.1) and all made generally better growth as the glucose concentration was raised. However, in three cases (*H.crustuliniforme*, *L.proxima* and *H.sacchariolens*) 1% glucose was clearly supra-optimal, and in one of these instances (*H.sacchariolens*) the best rate of linear growth was attained on 0. 1% glucose. These findings bear no obvious relationship to the different abilities of the fungi to form mycorrhizas on seedlings at different glucose levels (Table 5.1.4).

Fig 5.1.1
Linear growth of colonies of ectomycorrhizal fungi on plates of MMN agar supplemented with different concentrations of glucose.
(means of 3 replicate plates)



5.1.4. Effect of glucose concentration on fungal growth and mycorrhizal development II .

The foregoing experiment was repeated with minor modifications. MMN agar containing 0,0.01, 0.1 and 1 % glucose was prepared in boiling tubes, inoculated with nine fungi (*L.proxima* isolates 19 and 22, *H.crustuliniforme* isolates 4 and 8, *L.pubescens* isolates 4 and 6 , *T.fulvum* isolates 2 and 4 and *A.muscaria* isolate 5) and subsequently planted with aseptically germinated birch seedlings. Controls without fungal inoculum also were included giving ten sets of tubes in all. Incubation conditions were as before and the experiment was sampled after 12 weeks'growth of the plants (Tables 5.1.5 - 5.1.8).

As shown in Table 5.1.5, the mean number of root tips per seedling was significantly affected by glucose content of the agar; significantly more tips were produced at 0.1% and 1.0% glucose than with 0 or 0.01% glucose. This was true even in the absence of mycorrhizal inoculation and, in general, it was little affected by the type of fungus used. However, the number of root tips remained low at all glucose levels in the presence of *L.pubescens* (isolate 6) and, in contrast, was high throughout in the presence on *H.crustuliniforme* (isolate 4).

Overall, the percentage of root tips that became mycorrhizal increased with glucose concentration, but the pattern of variation was not identical to that for the development of root tips. Seedlings without glucose had usually a low percentage of mycorrhizal tips compared with that at all other glucose levels, and a further significant increase in mycorrhizal status occurred when glucose was raised from 0.01 or 0.1% to 1.0% (Table 5.1.6). Of all the inoculant fungi, only *L.proxima*[19] and *L.pubescens*[4] infected a substantial proportion of root tips in the absence of glucose and did not exhibit a major increase in mycorrhizal

Tables 5.1.5 - 5.1.8 Effect of glucose concentration in MMN agar on growth and mycorrhizal development of birch seedlings incubated for 12 wk and inoculated with different mycorrhizal fungi.(Means of 10 replicates).

Table 5.1.5
Mean number of root tips per seedling

Fungus	Glucose content 0%	0.01%	0.1%	1%	Row Mean		
Lpx19	15	26	48	42	32.2		
Lpx22	19	16	55	26	29.0		
Hc4	60	54	94	73	71.0		
Hc8	29	41	96	27	50.0	SED	7.80
Lp4	17	24	30	76	32.6	5%LSD	15.29
Lp6	11	7	13	25	14.9	1%LSD	20.09
Tf2	10	7	52	86	38.9	0.1%LSD	25.67
Tf4	13	8	32	71	31.1		
Am5	19	49	74	66	54.2		
Control	11	10	55	46	31.5		
Column Means	21.5	25.1	56.9	50.7			

SED	4.93
5%LSD	9.67
1%LSD	12.70
0.1%LSD	16.22

Table 5.1.6
Mean percentage of mycorrhizal tips per seedling

Fungus	Glucose content 0%	0.01%	0.1%	1%	Row Mean		
Lpx19	26.7	33.2	34.1	35.7	34.3		
Lpx22	13.5	30.6	23.2	29.4	24.2		
Hc4	8.3	31.1	60.5	30.8	32.7		
Hc8	8.6	25.1	26.0	25.6	20.9	SED	4.84
Lp4	26.7	33.3	34.1	35.7	32.5	5%LSD	9.49
Lp6	0	0	0.5	22.0	5.6	1%LSD	12.47
Tf2	12.7	28.4	31.6	31.2	26.0	0.1%LSD	15.93
Tf4	7.1	19.5	0.8	42.3	17.4		
Am5	0	0.2	2.9	23.7	6.7		
Control	-	-	-	-	-		
Column Means	10.4	20.6	21.4	27.8			

SED	3.06
5%LSD	5.99
1%LSD	7.88
0.1%LSD	10.07

Table 5.1.7

Shoot dry weight (mg)

Fungus	Glucose content 0%	0.01%	0.1%	1%	Row Mean		
Lpx19	27.8	53.3	148.7	43.3	67.5		
Lpx22	47.3	44.1	165.7	23.0	71.3		
Hc4	213.0	253.6	197.9	149.6	203.1		
Hc8	162.9	173.5	363.1	129.4	207.3	SED	23.32
Lp4	60.7	31.4	61.0	68.6	48.3	5%LSD	45.71
Lp6	23.5	31.4	61.0	68.6	48.3	1%LSD	60.07
Tf2	34.4	28.7	87.8	111.3	74.4	0.1%LSD	76.75
Tf4	40.6	24.0	106.1	122.3	74.1		
Am5	72.1	120.0	138.1	209.2	135.3		
Control	59.2	42.5	179.7	195.6	126.1		
Column Means	72.9	87.3	153.3	131.2			

SED	14.75
5%LSD	28.91
1%LSD	37.99

Table 5.1.8
Shoot length (mm)

Fungus	Glucose content 0%	0.01%	0.1%	1%	Row Mean		
Lpx19	40.7	38.1	101.0	22.9	50.7		
Lpx22	40.7	36.6	83.6	12.6	43.4		
Hc4	60.0	69.3	53.9	27.6	52.3		
Hc8	44.4	53.5	91.1	30.4	54.8	SED	7.53
Lp4	42.8	53.0	83.4	36.0	58.2	5%LSD	14.76
Lp6	26.7	32.6	84.8	45.4	46.0	1%LSD	19.40
Tf2	36.3	21.0	55.0	78.7	47.7		
Tf4	22.9	26.4	76.6	43.0	42.2		
Am5	16.1	59.0	42.3	35.2	38.1		
Control	46.0	54.1	82.9	90.7	68.4		
Column Means	37.3	44.0	75.2	44.2			

SED	4.76
5%LSD	9.33
0.1%LSD	15.66

development as glucose concentration was raised. Two isolates were used for each of four fungi, and the isolates sometimes behaved differently from one another. In particular, isolate 6 of *L.pubescens* infected few root tips at any but the highest glucose levels, whereas isolate 4 of *L.pubescens* infected one-quarter to one-third of tips at all glucose levels. Similarly, isolate 4 of *T.fulvum* infected relatively few tips except at the highest glucose level, whereas isolate 2 of *T.fulvum* infected about one-third of tips in all treatments except in the absence of glucose (Table 5.1.6). But these differences seemed to reflect mainly the deficiencies of particular isolates to form mycorrhizas (at least at low glucose levels), and the same general trends in mycorrhizal development with respect to glucose levels were seen in all cases.

Shoot dry weight increased progressively with glucose supplementation up to 0.1% but showed no further increase when the glucose concentration was raised to 1.0% (Table 5.1.7). This reflects closely the response of root numbers to glucose supplements (Table 5.1.5). Shoot weight also varied substantially between inoculation treatments when data for all glucose treatments levels were pooled. In this respect it is notable that shoot weights were significantly higher in the presence of both isolates of *H.crustuliniforme* than in all other treatments. Inspection of Table 5.1.7 shows that this effect was most apparent at low levels of glucose or in the absence of glucose; indeed, it was lost at the highest glucose level. It was not related to mycorrhizal development by *H.crustuliniforme*, as shown by comparison of Tables 5.1.6 and 5.1.7. Conversely, the combined data for all glucose treatments (Table 5.1.7) show that some inoculant fungi significantly reduced shoot weight relative to that of control (uninoculated) plants. Isolate 6 of *L.pubescens* had the largest inhibitory effect, but both isolates of *L.proxima* and both of *T.fulvum* also had somewhat inhibitory effects on shoot weights. These inhibitory effects were usually most

pronounced at the highest glucose levels, when the control plants grew well.

Shoot height (Table 5.1.8) was a less sensitive indicator of plant response to inoculation or to glucose supplementation than was shoot weight. In fact, the control plants were tallest overall; isolates of *H.crustuliniforme* had little or no effect on shoot height, despite having large effects on shoot weight; but data for shoot height again suggested that inoculation with isolates of *L.proxima* and *T.fulvum* reduced plant growth relative to that in controls.

Fungal growth rates on plates of agar with different glucose contents are shown in Fig 5.1.2.

For *L.proxima* isolates 19 and 22 there was no significant difference in radial growth with increase in glucose content from 0 to 1.0%, although at 0 and 0.01% glucose the fungal mycelium was very sparse; similar results were obtained in the previous experiment. These results broadly reflect those for effects of glucose on mycorrhizal development by the isolates of *L.proxima* (Table 5.1.6).

Isolates of all the other fungi showed faster extension growth in the presence than in the absence of glucose and often (though not in the case of *H.crustuliniforme*) showed progresseve increases in extension growth as the glucose concentration was raised progressively. At one percent concentration, however, glucose was evidently supra-optimal for extension growth by both isolates of *H.crustuliniforme*. A major interpretational problem with the results in Fig 5.1.2 is to decide whether effects of glucose on the initiation of colony growth are more or less meaningful than effects of glucose on subsequent rates of growth. For example, in the case of *L.proxima* isolate 19, initial rates of colony extension were slowest at the lowest glucose levels, but these low glucose

Fig 5.1.2
Linear growth of colonies of ectomycorrhizal fungi on plates of MMN agar supplemented with different concentrations of glucose.
(means of 3 replicate plates)

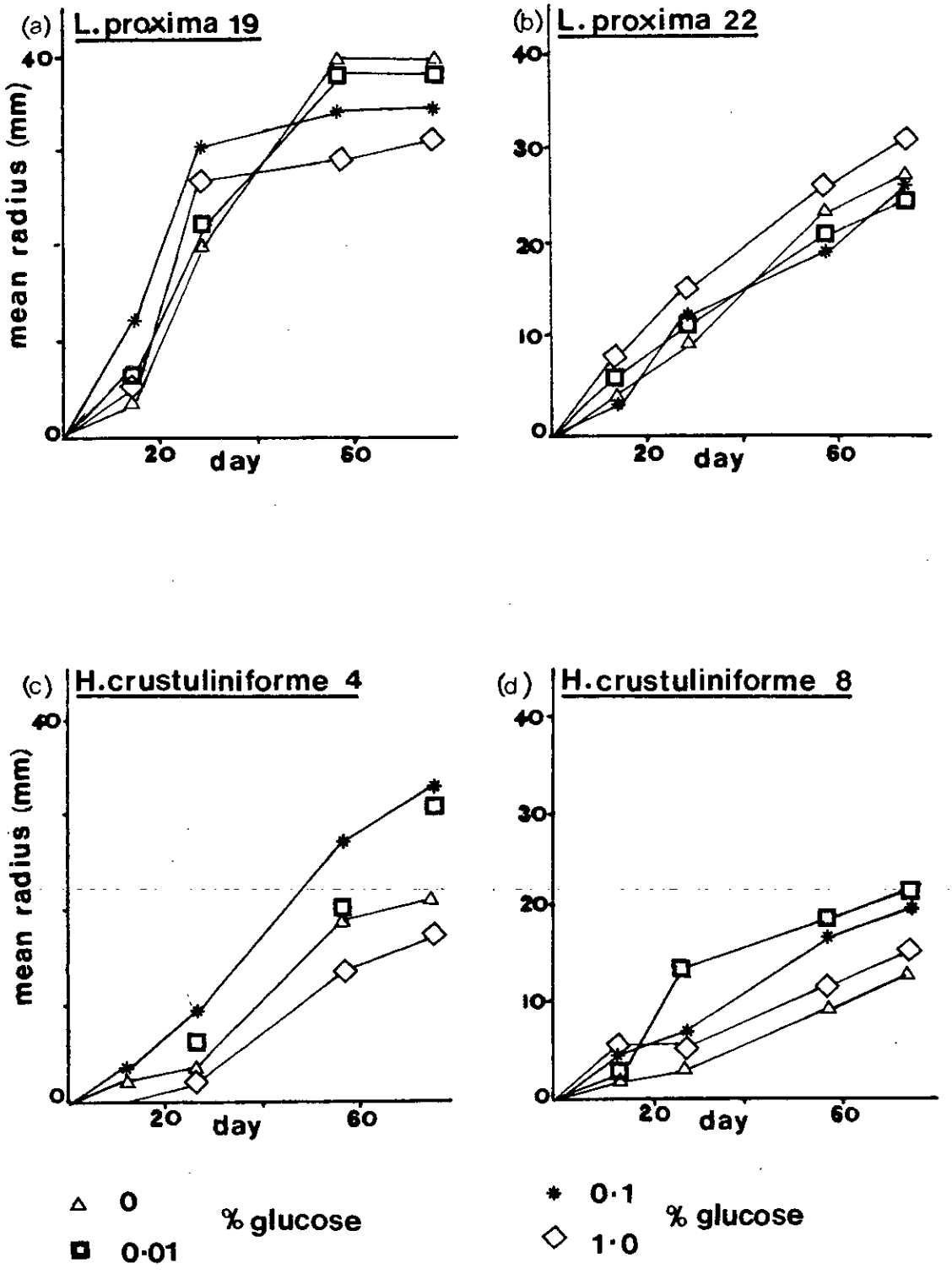


Fig 5.1.2 continued

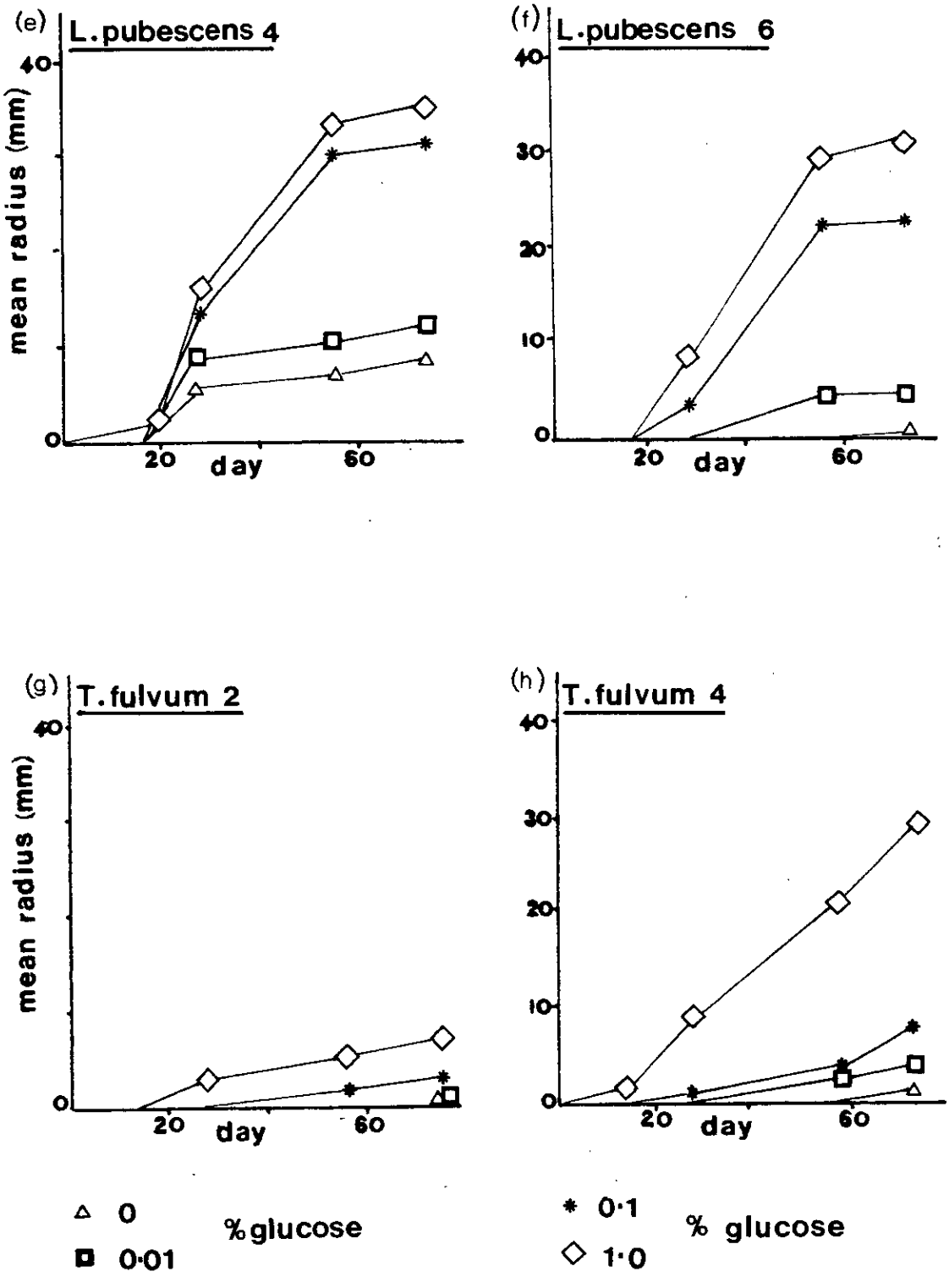
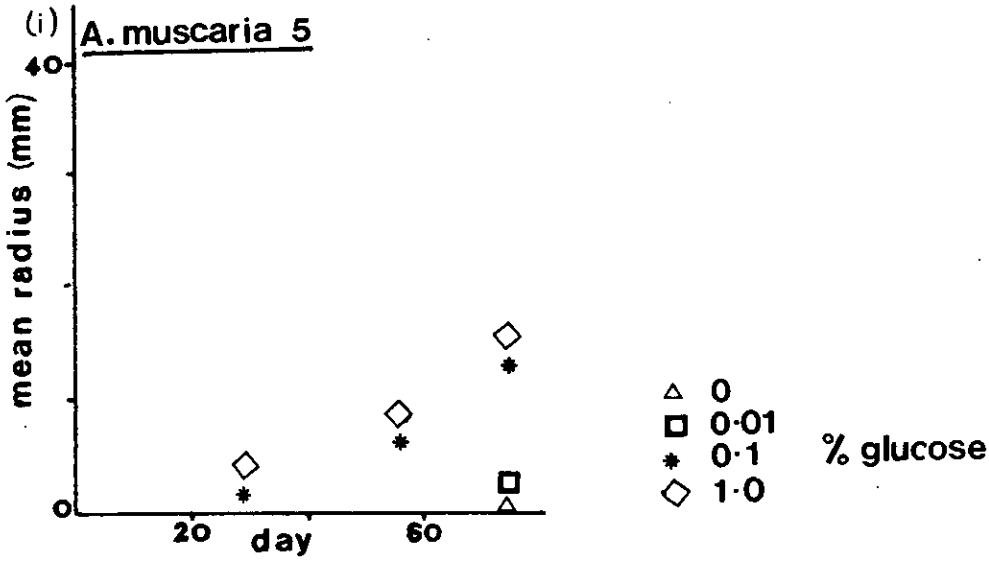


Fig 5.1.2 continued



levels sustained colony extension for longer than did high glucose levels. Comparison of Fig 5.1.2 with Table 5.1.6 suggests that early growth responses of the fungi to glucose may be the most meaningful in terms of successful mycorrhizal development. In this respect it must be noted that the fungi were inoculated onto the slopes of agar 2 weeks before seedlings were added. Thus *L.proxima* isolates 19 and 22 infected a similar proportion of root tips at all glucose levels (Table 5.1.6), and these isolates also grew initially on agars at all glucose levels. The two isolates of *H.crustuliniforme*, however, infected low percentages of root tips in the absence of glucose, and higher proportions at higher glucose levels, which is at least partly related to their long lag phase of extension growth in the absence of glucose (especially in the case of isolate 8). *L.pubescens* isolate 4 began to grow at all glucose levels, even if it's rate slowed markedly thereafter at the glucose levels, and it infected well at all glucose levels. In contrast, isolate 6 of *L.pubescens* started to grow rapidly only at the highest glucose level, at which it formed most mycorrhizas. Isolate 4 of *T.fulvum* showed similar behaviour to isolate 6 of *L.pubescens*, and so did the single isolate (5) of *Amanita muscaria*. The only major anomaly concerns isolate 2 of *T.fulvum*, which grew poorly on all media and yet still formed mycorrhizas on a considerable proportion of seedling roots.

Four fungi were included in both this and the previous experiment. Of these four, isolate 4 of *L.pubescens* and isolate 5 of *A.muscaria* showed similar trends in both experiments with regard to growth rate on agar, though not with regard to mycorrhizal development. *L.proxima* 19 showed similar trends for both growth rate on agar and mycorrhizal development. *H.crustuliniforme* 4 showed somewhat different trends in both respects between the experiments. Overall, there was substantially more mycorrhizal development in the first than in the second experiment, and the general pattern of mycorrhizal development differed,

there being more mycorrhizas in the absence of glucose in experiment 1 than in experiment 2, and the stimulatory effect of glucose on mycorrhizal development occurring only at high glucose levels in the first experiment, compared with at low glucose levels in the second.

5.1.5. Effects of reductions in the concentration of N or P on fungal growth and mycorrhizal development.

In this experiment, birch seedlings were grown on slopes of agar in boiling tubes as described in Section 2.1.2. The agar (MMN with 1.0% glucose) was used unaltered or with either nitrogen or phosphorus at one- tenth of standard concentration (see Section 5.1.2). Each tube was inoculated with one of the eight mycorrhizal fungi listed in Table 5.1.9 or with a sterile agar disc (control), then an aseptically germinated birch seedling was planted. The seven replicate tubes of each treatment were arranged in randomised blocks beneath four 100W "Grolux" strip lights and maintained at 23°C. After 12 weeks'incubation, the seedlings were assessed for shoot height, shoot dry weight, total number of root tips, proportion of tips that were mycorrhizal and root dry weight. (Tables 5.1.9 - 5.1.14). The results, subjected to analysis of variance, revealed that, overall, the plants grown at standard concentrations of N and P were significantly taller and heavier that those at one-tenth P concentration, and these plants in turn were taller and heavier than at one-tenth N concentration. Root dry weights showed a similar trend, as did the total number of root tips. The root:shoot ratio (on a dry weight basis) did not differ significantly across the mineral nutrition treatments. The percentage of root tips that became mycorrhizal (Table 5.1.10) also was markedly (P=0.01) affected by differences in mineral nutrition. The highest percentage infection occurred on the "standard" medium; significantly lower percentages occurred on the low-P medium, and again significantly the lowest percentages occurred on the low-N medium.

Inspection of Table 5.1.10 shows that all of the inoculant fungi developed mycorrhizas in the experiment. The only instance in which no mycorrhizas were seen was with *Lactarius pubescens* 6 on the low-N medium.

Tables 5.1.9 - 5.1.14 Effect of different levels of nitrogen and phosphorus on the growth and mycorrhizal development of birch seedlings inoculated with different mycorrhizal fungi and incubated for 12 wk.(Means of 10 replicates).

Table 5.1.9
Mean number of root tips per seedling

Fungus	Normal	Reduced P	Reduced N	Row Mean		
Lpx19	31.6	49.1	17.7	35.9		
Lpx22	21.0	19.7	13.7	18.1		
Hc4	45.6	24.7	13.7	28.8		
Hc8	43.4	23.3	27.5	31.0		
Lp4	63.1	30.8	34.7	42.0	5%LSD	13.54
Lp6	77.6	59.3	51.9	62.9	1%LSD	18.01
Am5	62.0	73.4	54.3	64.7		
Am7	63.4	55.1	43.3	54.0		
Control	73.9	61.0	60.6	66.5		
Column Means	53.3	44.2	36.4			

SED	3.91
5%LSD	7.82
1%LSD	10.40

Table 5.1.10
Mean percentage of mycorrhizal tips per seedling

Fungus	Normal	Reduced P	Reduced N	Row Mean		
Lpx19	19.1	14.2	4.8	12.7		
Lpx22	18.2	15.2	9.9	14.4		
Hc4	26.3	25.0	14.6	22.2		
Hc8	28.9	27.6	3.1	19.9	SED	4.01
Lp4	34.2	13.9	7.2	18.2	5%LSD	7.86
Lp6	13.4	4.0	0	5.6	1%LSD	10.33
Am5	10.8	3.7	3.3	5.9	0.1%LSD	13.20
Am7	19.5	3.9	6.0	9.8		
Control	-	-	-	-		
Column Means	18.84	11.84	5.60			

SED	2.32
5%LSD	4.54
1%LSD	5.97
0.1%LSD	7.63

Moreover, the fungi in general followed a similar trend, with lower percentage infection on the low-N and low-P media than on the standard medium. However, it is notable that both isolates of *Laccaria proxima* and both isolates of *Hebeloma crustuliniforme* were less affected by a reduction of P content than by a reduction of N-content of the medium. In contrast, the infectivity of both isolates of *L.pubescens* and both of *Amanita muscaria* was markedly reduced on the low-P medium compared with on the standard medium - nearly as much reduced on the low-P as on the low-N medium.

The different mycorrhizal fungi also influenced plant growth to different degrees (Tables 5.1.11 - 5.1.14). All significantly reduced seedling height and dry weight relative to that in the absence of inoculation, when the effect is considered for mineral nutrient treatments as a whole (see final columns of Tables 5.1.11 and 5.1.13). The least effect, however, was caused by *L.pubescens* 6 and *A.muscaria* 5 - the two isolates that were the least infective (Table 5.1.10). Inspection of the body of each table shows that the growth-reducing effects of mycorrhizal inoculation occurred in all mineral nutrient treatments, with the sole exception that *A.muscaria* 5 tended to enhance shoot growth on the low-P medium (Table 5.1.11). Nevertheless, in proportional terms the growth-reducing effects of the fungi were greatest on the standard medium. Another interesting feature of the results for shoot growth is that this was generally reduced the most when the mycorrhizal fungus established mycorrhizas on a high proportion of root tips, although this relationship did not always hold. Root growth was reduced less that shoot growth by inoculation, an effect seen most clearly in the higher root:shoot ratios of inoculated than of uninoculated plants (final column of Table 5.1.12). Lastly, the tables suggest that the four isolates typically regarded as representing late-stage fungi in mycorrhizal successions (i.e. the isolates of *L.pubescens* and *A.muscaria*) had generally lesser effects on all components of

Table 5.1.11
Shoot height (mm)

Fungus	Normal	Reduced P	Reduced N	Row Mean		
Lpx19	2.7	2.8	1.3	2.22		
Lpx22	1.4	1.6	0.7	1.26		
Hc4	2.7	2.0	1.1	1.85		
Hc8	2.9	2.3	1.7	2.32		
Lp4	3.4	3.2	2.6	3.08	SED	0.46
Lp6	6.6	3.9	3.1	4.55	5%LSD	0.89
Am5	4.7	6.4	3.3	4.80	1%LSD	1.18
Am7	4.9	3.2	2.4	3.54	0.1%LSD	1.50
Control	9.9	5.5	3.4	6.25		
Column Means	4.37	3.41	2.18			

SED	0.264
5%LSD	0.52
1%LSD	0.68
0.1%LSD	0.88

Table 5.1.12
Root : Shoot ratio (dry weights)

Fungus	Normal	Reduced P	Reduced N	Row Mean		
Lpx19	0.74	0.51	0.82	0.66		
Lpx22	0.57	0.78	0.81	0.71		
Hc4	0.71	1.28	0.62	0.81		
Hc8	0.44	0.60	0.53	0.58	SED	0.48
Lp4	0.80	4.10	0.50	1.75	5%LSD	0.95
Lp6	0.34	0.51	0.58	0.52	1%LSD	1.25
Am5	0.47	0.35	0.69	0.51		
Am7	0.48	0.57	0.52	0.52		
Control	0.19	0.34	0.41	0.33		
Column Means	0.51	1.00	0.62			

SED	0.281
5%LSD	-

Table 5.1.13
Shoot dry weight (mg)

Fungus	Normal	Reduced P	Reduced N	Row Mean		
Lpx19	25.5	84.8	17.0	58.5		
Lpx22	19.0	18.0	8.6	16.5		
Hc4	45.6	25.1	12.0	27.0		
Hc8	69.1	27.4	13.8	36.5	SED	18.56
Lp4	79.3	23.0	35.4	44.2	5%LSD	36.38
Lp6	237.5	150.0	49.7	144.2	1%LSD	47.81
Am5	153.9	207.4	46.9	136.0	0.1%LSD	61.08
Am7	115.9	85.0	41.3	80.7		
Control	421.0	193.4	89.9	233.5		
Column Means	128.4	95.6	35.0			

SED	10.72
5%LSD	21.01
1%LSD	27.61
0.1%LSD	35.28

Table 5.1.14
Root dry weight (mg)

Fungus	Normal	Reduced P	Reduced N	Row Mean		
Lpx19	15.5	60.1	11.4	28.9		
Lpx22	9.7	13.5	6.3	10.2		
Hc4	28.3	22.3	7.7	19.2		
Hc8	31.7	14.0	6.8	17.4	SED	7.09
Lp4	36.7	21.8	18.0	24.8	5%LSD	13.89
Lp6	79.7	65.0	24.9	55.9	1%LSD	18.26
Am5	66.3	72.3	30.6	56.4	0.1%LSD	23.33
Control	86.2	48.9	37.0	56.8		
Column Means	44.9	40.0	18.1			

SED	4.09
5%LSD	8.02
1%LSD	10.53
0.1%LSD	13.46

Tables 5.1.15 - 5.1.18
Comparison of the growth and mycorrhizal development of birch seedlings inoculated with different mycorrhizal fungi in experiments involving different concentrations of glucose and mineral nutrients in MMN agar.

Table 5.1.15
Mean number of root tips per seedling

Fungus	Glucose	Mineral Nutrition	Row Mean		
Lpx19	40.6	31.6	36.9		
Lpx22	25.9	21.0	23.4		
Hc4	72.9	45.6	59.2		
Hc8	26.7	43.4	69.6	SED	20.3
Lp4	42.1	63.1	52.6	5%LSD	40.2
Lp6	29.1	77.6	53.4		
Am5	65.7	62.0	63.9		
Control	45.4	77.9	61.6		
Column Means	43.7	52.8			
	SED	10.15			
	5%LSD	-			

Table 5.1.16
Mean percentage of mycorrhizal tips per seedling

Fungus	Glucose	Mineral Nutrition	Row Mean		
Lpx19	35.6	19.1	27.4		
Lpx22	29.4	18.2	23.8		
Hc4	30.8	26.3	28.6		
Hc8	27.0	28.9	28.0	SED	6.38
Lp4	35.7	34.6	35.1	5%LSD	12.63
Lp6	22.0	13.4	17.6	1%LSD	16.70
Am5	23.7	10.8	17.3		
Control	-	-	-		
Column Means	25.5	18.9			
	SED	3.19			
	5%LSD	6.32			

Table 5.1.17

Shoot dry weight (mg)

Fungus	Glucose	Mineral Nutrition	Row Mean		
Lpx19	44.3	25.5	35.5		
Lpx22	23.0	19.0	23.9		
Hc4	149.7	45.6	76.2		
Hc8	129.4	69.1	99.3	SED	29.48
Lp4	204.5	79.3	143.4	5%LSD	58.37
Lp6	68.6	237.5	124.9	1%LSD	77.15
Am5	210.8	153.9	182.1	.1%LSD	99.44
Control	199.4	421.0	307.4		
Column Mean	124.3	123.2			

SED 14.74
5%LSD -

Table 5.1.18
Shoot length (mm)

Fungus	Glucose	Mineral Nutrition	Row Mean		
Lpx19	39.4	27.1	33.3		
Lpx22	12.6	14.1	13.4		
Hc4	27.6	26.7	27.1		
Hc8	30.4	29.4	29.9	SED	5.81
Lp4	48.9	34.4	41.6	5%LSD	11.50
Lp6	45.4	66.3	54.1	1%LSD	15.20
Am5	35.2	40.6	40.9	.1%LSD	19.60
Control	90.7	98.8	94.1		
Column Mean	41.0	42.6			

SED 2.91
5%LSD -

Fig 5.1.3
Linear growth of colonies of ectomycorrhizal fungi on plates of MMN agar supplemented with different concentrations of N and P.
(means of 3 replicate plates)

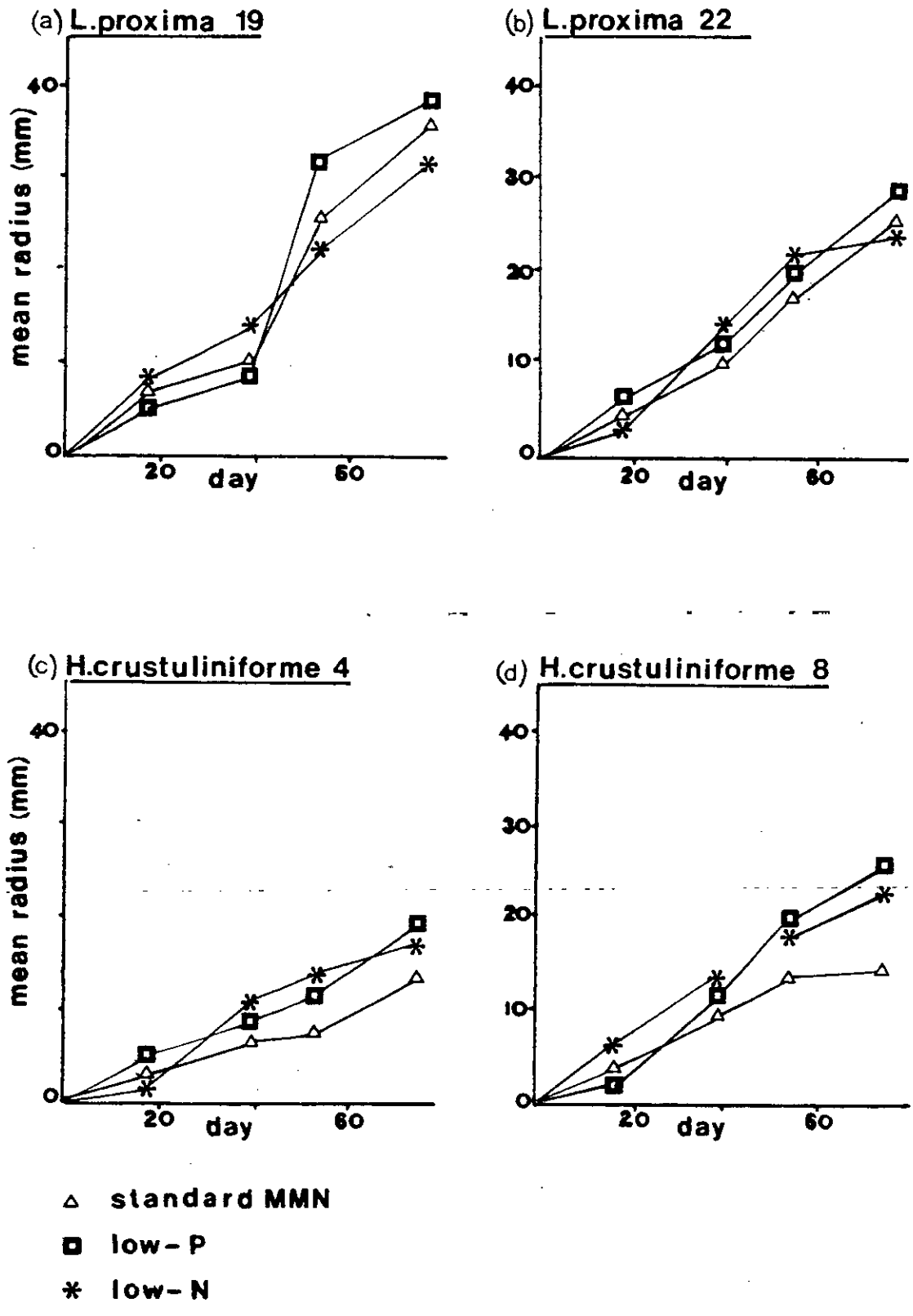
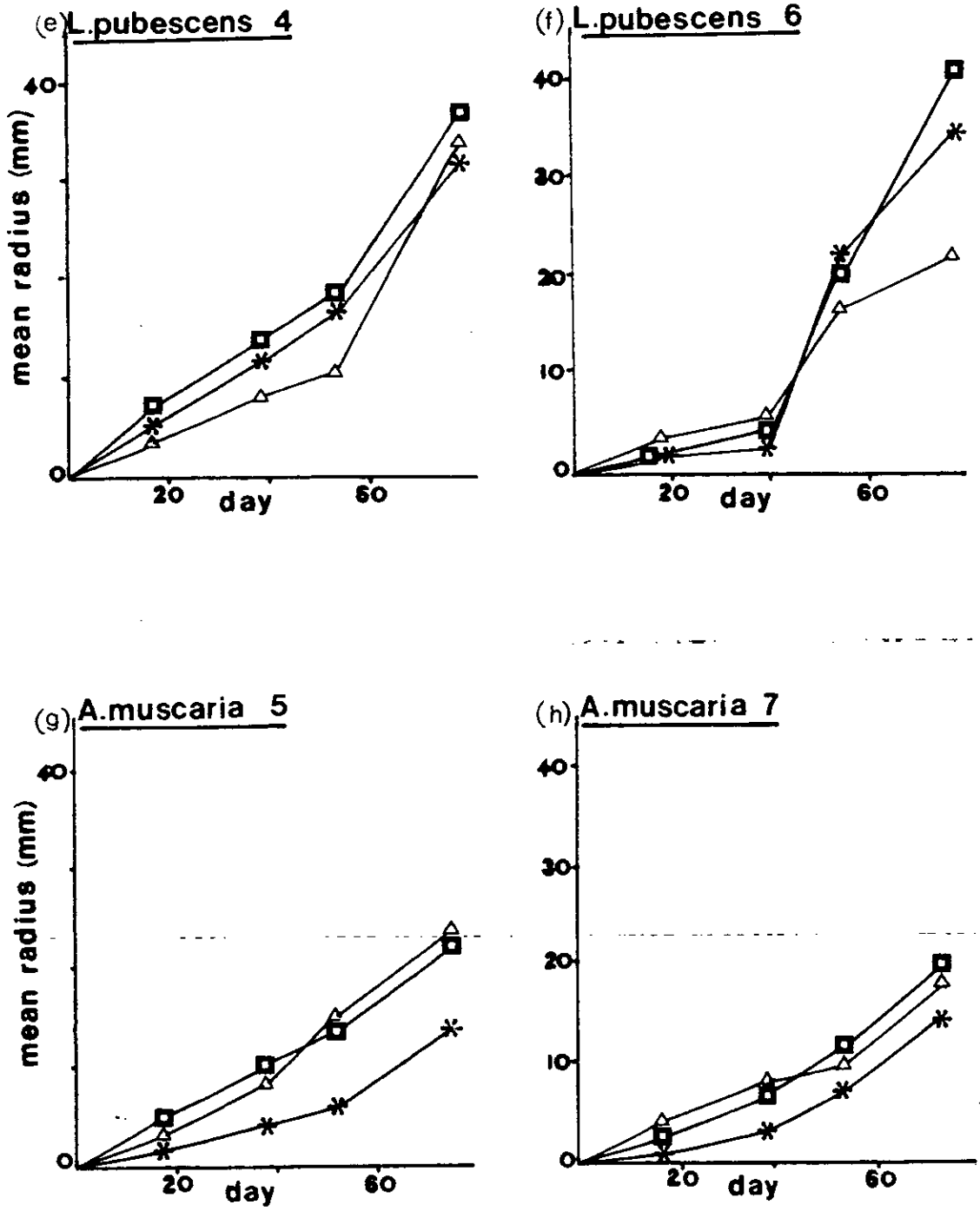


Fig 5.1.3 continued



root or shoot growth than did the four isolates of early-stage fungi (i.e. *H.crustuliniforme* and *L.proxima*).

The results for the standard mineral nutrient medium in this experiment are directly comparable with those for the "1% glucose" treatment in the previous experiment (Tables 5.1.5 - 5.1.8). The relevant data are assembled in Tables 5.1.15 - 5.1.18. From these it is seen that the fungi established mycorrhizas more consistently in the previous experiment that in the present one, and that their effects on shoot weight tended to differ in the two experiments. Only the two isolates of *L.proxima* caused consistent marked reductions in shoot growth in the two experiments, and they had much lesser effects on root growth as evidenced by the numbers of root tips.

All of the fungal isolates used in the present experiment grew on petri dishes containing the normal MMN agar and also on agars with either one-tenth phosphorus or one-tenth nitrogen . Colony extension rates on these three media, as shown in Fig 5.1.3, were not significantly different although the colonies were most dense on the standard MMN medium. The radial growth rates in this experiment were similar to those in a previous experiment involving MMN agar with 1% glucose (Fig 5.1.2).

5.1.6. Discussion

As noted earlier, the dependence of mycorrhizal fungi on the host plant for a supply of carbohydrate has been well documented (Melin & Nilsson, 1957; Lewis & Harley, 1965 a,b,c; Hacskaylo, 1973; Harley, 1978). The first two experiments in this section were designed to test the effect of exogenously supplied glucose on mycorrhizal formation and on fungal growth in axenic culture conditions.

In both of these experiments, although they involved slightly different growth conditions and tested a different range of species and isolates, plant growth tended to be reduced by the highest level of glucose in the medium (1%) but plant growth was poorest when glucose was absent altogether from the medium. Glucose *per se* probably directly affected plant growth by supplementing the supply of carbohydrate available from photosynthesis. It may, however, also have had indirect effects - for example through alteration of the osmotic potential of the medium.

Root development, as assessed by number of tips per plant, was not consistent between the two experiments. In the first experiment glucose did not affect the number of root tips but the fungal inocula did so, and especially large root systems formed in association with *L.pubescens* 4. In the second experiment, however, fungal inocula did not affect the number of root tips but this parameter increased with increasing glucose supply.

In both experiments, the late-stage species *A.muscaria* 5, formed mycorrhizas considerably better when more glucose was present in the medium (1%), whereas mycorrhizal development by *L.proxima* 19 and *H.crustuliniforme* 4 tended to be unaffected by increasing glucose levels. This could suggest that late stage species require more carbohydrate for mycorrhizal development than is available from photosynthesis by very young seedlings. In the second experiment there was also variability between the two isolates of the species used; in particular few mycorrhizas were formed by *L.pubescens* 6 and *T.fulvum* 4 compared to *L.pubescens* 4 and *T.fulvum* 2. Such differences in behaviour between isolates were perhaps related to length of storage in culture but suggest the need for caution in interpreting the effects of carbohydrates on mycorrhizal development.

The third experiment, which in a similar system to the second glucose experiment made phosphorus or nitrogen available to fungi and seedlings at a standard or one-tenth concentration, also showed that there could be marked variation between two isolates of the same species and again the isolates of *L.pubescens* and *A.muscaria* highlighted these differences. Overall, seedling height and dry weight were less on agar containing one-tenth than "standard" phosphorus and similarly less when nitrogen was available at only one-tenth concentration; these trends were seen irrespective of the inoculant fungi used. There was no evidence that the late stage species were better adapted than early stage species to conditions of low nitrogen or phosphorus; indeed, the early stage species (*L.proxima* 19 and 22 and *H.crustuliniforme* 4 and 8) and *L.pubescens* 4, tended to form more mycorrhizas at low levels of nitrogen or phosphorus than did *L.pubescens* 6 or the two isolates of *A.muscaria*.

The results of the second glucose experiment and the mineral nutrition experiment can be directly compared as the same agar, fungal isolates and physical conditions were used for each. Plant growth was equivalent in the two experiments, but mycorrhizal development was significantly greater ($P = 0.05$) in the glucose than in the mineral nutrition experiment. In particular, this was true for the isolates of *L.proxima*, *L.pubescens* and *A.muscaria*, suggesting that it was not related to the efficacy of the inoculum *per se*, but rather to relatively minor, undetected differences in the conditions in which the experiments were performed.

Overall, the approach used in the experiments described above showed little promise as a means of detecting physiological or behavioural differences between mycorrhizal fungi from different stages in reported successions. Any such differences that might exist were masked by differences between experiments or differences between individual isolates.

5.2. Fungal interactions on agar.

5.2.1. Introduction

Previous studies have investigated interactions between fungi and bacteria (Bowen & Theodorou, 1979) and between ectomycorrhizal fungi and plant pathogens (Marx,1968,1970), both in vivo and in vitro. However, little attention has been given to competitive or antagonistic interactions between mycorrhizal fungi, and this may be important in relation to the colonisation of root systems from mixed inocula in soil and in relation to the possible replacement of one mycorrhizal type by another in individual parts of a root system. Trappe (1977) has pointed out that even young seedling trees in field conditions can bear mycorrhizas of at least two different fungal species, and often as many as six mycorrhizal types can be found on the roots of seedling trees in glasshouse or field sites (Fleming,1983 a; Fox,1983). On the other hand, Fox (1983) found that mixtures of spore inocula of *Laccaria proxima* and *Hebeloma crustuliniforme* applied to soil subsequently planted to birch seedlings often resulted in the exclusive establishment of one or other of these species on the seedling root systems, but whether this was due to chance or to competitive or antagonistic interactions was not determined.

In the experiments in this section, an attempt was made to assess the interactions of a range of mycorrhizal fungi both on agar and in liquid culture as a possible basis for predicting the competitive interactions of these fungi in vivo.

5.2.2. Pairing of various mycorrhizal fungi on agar.

Plates of half-strength Potato dextrose agar were prepared and inoculated with two 6mm diameter agar discs of fungi, spaced 2 cm apart near the centre of each plate. The fungi were *Thelephora terrestris* 1, *Laccaria proxima* 19, *Hebeloma crustuliniforme* 4, *H.sacchariolens* 4, *Lactarius pubescens* 4, *Leccinum scabrum* 1, *Tricholoma fulvum* 4 and *Amanita muscaria* 5. Each was paired with itself and with all others factorially, with three replicate plates per treatment. On four occasions during 8 weeks' incubation at 20° C, the shapes of the colonies were traced on to acetate sheets and transferred to paper.

The types of interaction observed were classified into three main types, as follows.

1) "Deadlock"(D) , in which the colonies grew together and formed a common boundary, with no further extension of either into the other.

2) "Merging"(M), in which the colonies grew towards each other and intermingled along the common boundary.

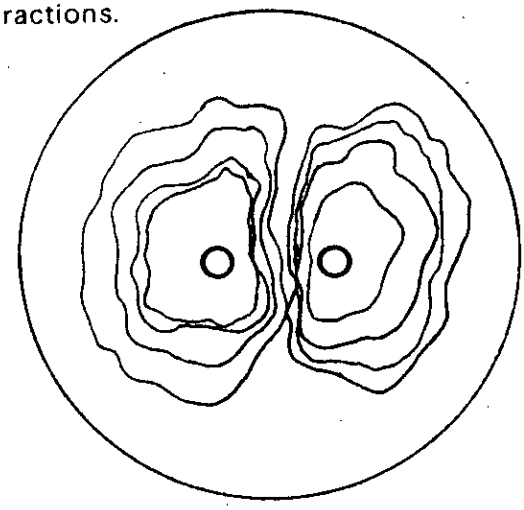
3) Inhibition (I) of growth of one colony only, before contact with the other colony.

The patterns of growth for each combination are summarised in the table below where, for the purpose of denoting inhibition, the fungi are referred to as "donors" or "recipients". An example of each pattern is shown in Fig 5.2.1.

Fig 5.2.1
Tracings of representative pairings of ectomycorrhizal fungi on agar plates, showing 3 types of interactions.

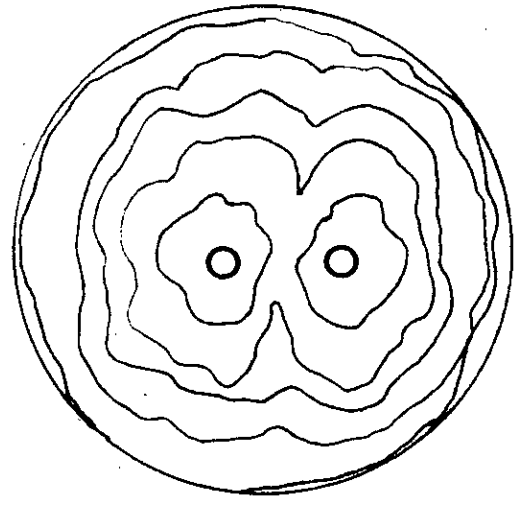
Mutual inhibition

(e.g. A.musc x T.fulv)



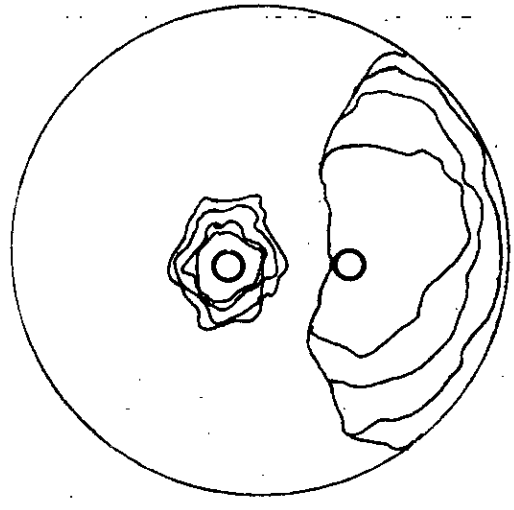
Merging of colonies

(e.g. L.prox x L.prox)



Inhibition of one colony

(e.g. H.sacc x L.prox)



Interactions between paired isolates.

"recipient colony" *	"Donor colony" * Thel	Lacc	H.crust	H.sacc	Lact	Lecc	Trich	Amanita
Thel	M	M	M	M	M	M	M	M
Lacc		M	I	I	M	M	M	M
H.crust			D	D	M	M	M	M
H.sacc				D	M	M	D	D
Lact					M	M	D	D
Lecc						M	D	D
Trich							M	D
Amanita								M

* Donor colonies elicited on the recipient colonies the responses shown, namely inhibition, deadlock or merging.

In the whole series of combinations, clear evidence of inhibition of one fungus by another was seen only when *H.crustuliniforme* or *H.sacchariolens* was opposed to *L.proxima*. This type of interaction was, therefore, studied further,using a wider range of species and isolates, as follows.

5.2.3. Interactions between isolates of *Hebeloma* and *Laccaria* species on agar plates

Laccaria proxima isolates 19 and 22, and *L.laccata* isolate 1, were paired on agar, as before, with *Hebeloma sacchariolens* isolates 4 and 6, *H.crustuliniforme* isolates 4,8 and 13, *H.subsaponaceum* isolates 2 and 3, *H.mesophaeum* isolate 1 and *Hebeloma* sp. isolate 3.

After 10 wk incubation on half-strength PDA at 20°C, the colony radii were measured in two directions - towards and away from the other colony. The extent of growth towards compared with away from the other colony was calculated, and means for the treatments are shown in Table 5.2.1.

The degree of growth change towards compared with away from the neighbouring colonies ranged from +14% (*H.sacchariolens* 4 being slightly stimulated as it grew towards *L.proxima* 19), down to -71% when growth of *L.proxima* 19 was reduced as it grew towards *H.sacchariolens* 6. Overall, growth of fungi towards other colonies was only 71% of that away from other colonies, and paired inocula of any one isolate (i.e. self x self) caused little mutual inhibition. The data for self x self pairings are boxed in Table 5.2.1, as they provide a basis of comparison for the non-self pairings.

By using the degree of growth-reduction in self-self pairings as a baseline, it seemed that growth of *H.sacchariolens* 6 was unaffected by the presence of any other isolate but *H.sacchariolens* 6 markedly affected the growth of almost all other isolates (except *H.crustuliniforme* 8). It is notable that this strongly inhibitory effect of *H.sacchariolens* 6 occurred despite the fact that this fungus formed very small colonies that rarely exceeded 2 mm radius , the only exception being that it reached 5 mm radius when paired with itself.The inhibitory effect of this fungus is shown in Fig 5.2.2.

H.sacchariolens 4 similarly inhibited the growth of all other fungi except *H.sacchariolens* 6 and *H.crustuliniforme* 13. Isolates of *Laccaria* spp. were particularly sensitive to inhibition by *H.sacchariolens* 4 as also to isolate 6 of this species .

The three isolates of *H.crustuliniforme* had relatively slight effects, if any, on growth by most other *Hebeloma* species but substantially reduced the growth of *H.mesophaeum* 1 and all isolates of *Laccaria*. The other Hebelomas (*Hebeloma* sp.3, *H.subsaponaceum* 2 and 3 and *H.mesophaeum* 1) similarly had

Table 5.2.1
Mean ratio of radial growth facing the centre of the plate:side of plate

"recipient colony"

"donor colony"	Hs6	Hs4	Hc4	Hc8	Hc13	Hsp3	Hb2	Hb3	Hm1	L19	L22	L11
Hs6	[0.89]	0.37	0.40	0.66	0.45	0.52	0.53	0.44	0.65	0.29	0.35	0.41
Hs4	0.91	[0.87]	0.67	0.61	0.79	0.59	0.59	0.63	0.50	0.33	0.34	0.45
Hc4	0.87	0.85	[0.92]	0.73	0.91	0.75	0.72	0.88	0.41	0.32	0.30	0.65
Hc8	0.88	0.73	0.73	[0.72]	0.65	0.54	0.72	0.75	0.46	0.56	0.42	0.34
Hc13	0.91	0.91	0.60	0.64	[0.73]	0.70	0.81	0.72	0.48	0.52	0.47	0.43
Hsp3	0.96	0.77	0.63	0.57	0.89	[0.79]	0.78	0.81	0.63	0.69	0.51	0.41
Hb2	1.10	0.68	0.81	0.75	0.69	0.65	[0.94]	0.79	0.69	0.70	0.52	0.31
Hb3	1.00	1.07	0.77	0.79	0.62	0.94	0.74	[0.86]	0.72	0.60	0.68	0.69
Hm1	0.79	0.81	0.79	0.81	0.63	0.62	0.67	0.69	[0.89]	0.63	0.73	0.50
L19	1.08	1.14	0.80	0.80	0.67	0.70	0.86	0.73	0.92	[0.96]	0.78	0.86
L22	0.91	0.87	0.83	0.73	0.69	0.77	0.69	0.70	0.90	0.81	[0.85]	0.79
L11	0.95	1.04	0.88	0.74	1.07	0.63	0.83	1.01	1.08	1.00	0.91	[0.90]

Hs = *Hebeloma saccrariolens* ; Hc = *H.crustuliniforme*
Hsp = *Hebeloma* sp. ; Hb = *H.subsaponaceum*
Hm = *H.mesophaeum*
L = *Laccaria proxima* ; Ll = *L.laccata*

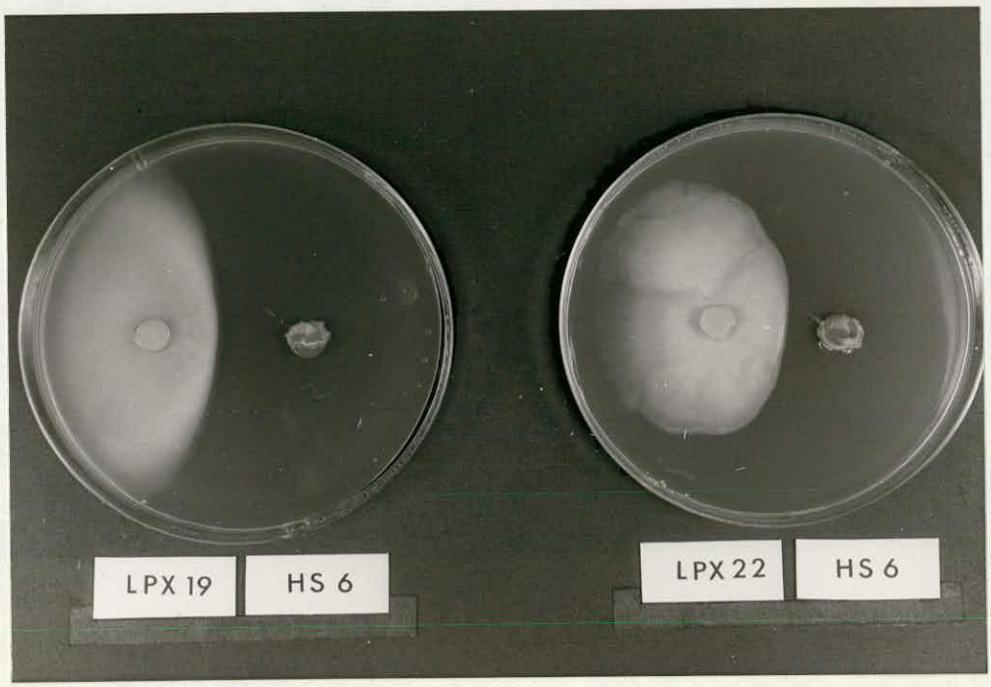
[ ] = self x self pairings

Overall mean = 0.71

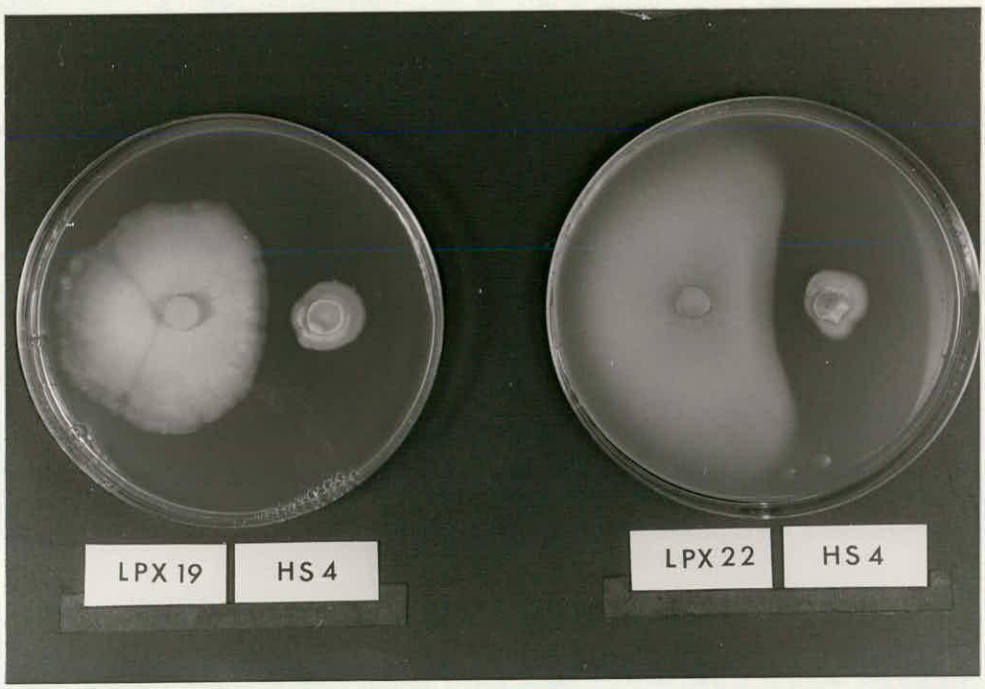
= ratios below overall mean

Fig 5.2.2
Examples of patterns of interaction between colonies of *Laccaria proxima* (Lx.) and *Hebeloma sacchariolens* (H.s) or *H.crustuliniforme* (H.c) and in "self x self" pairings.

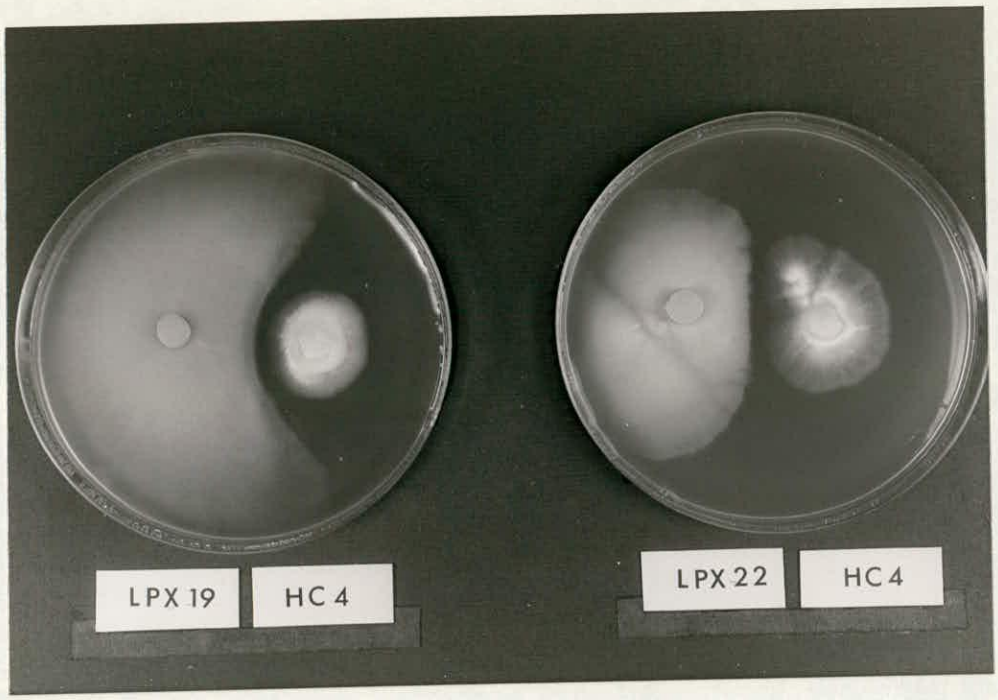
a) Lx19 x Hs6 ; Lx22 x Hs6



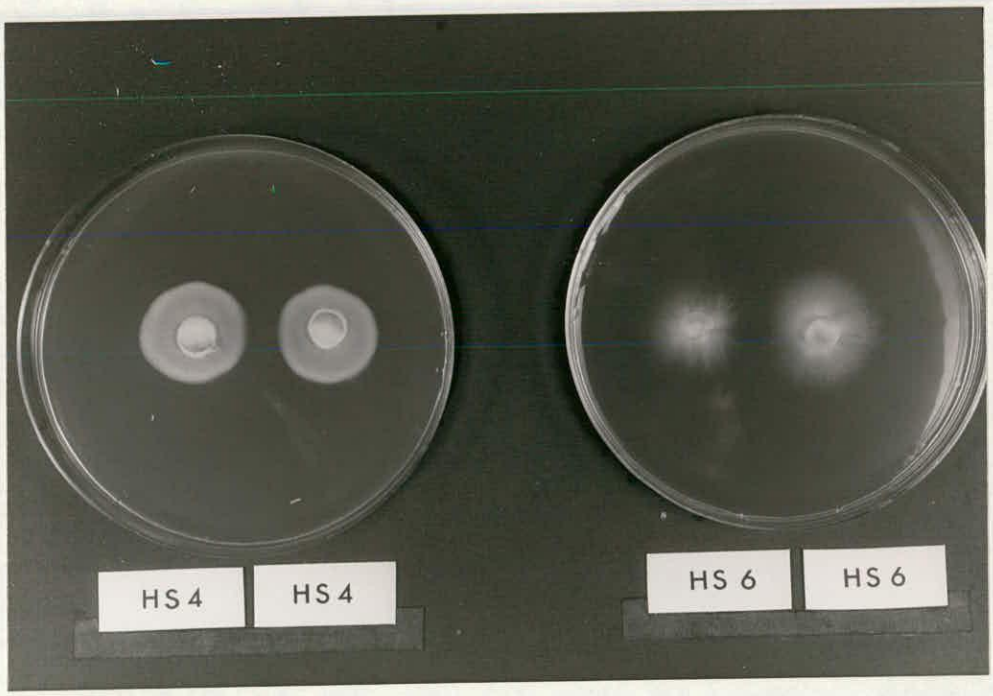
b) Lx19 x Hs4 ; Lx22 x Hs4



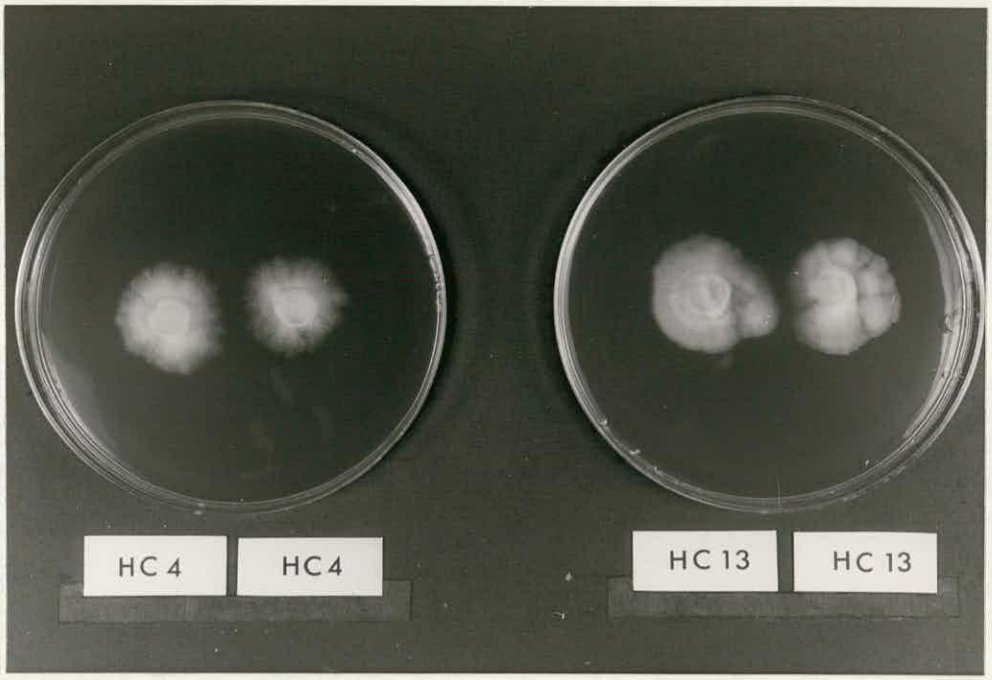
c) Lx19 x Hc4 ; Lx22 x Hc4



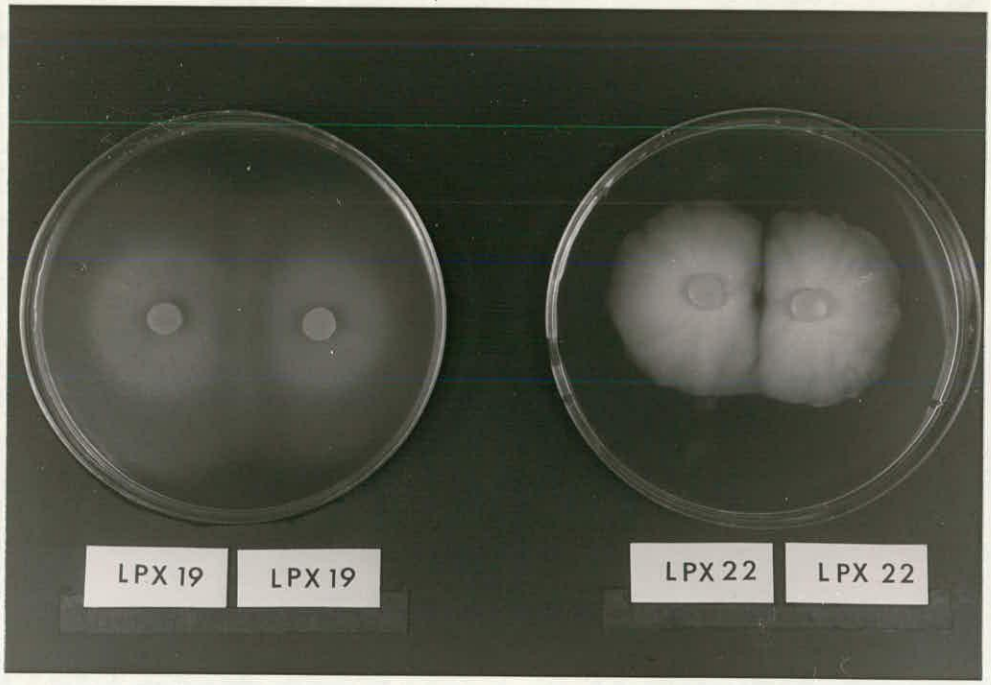
d) Hs6 x Hs6 ; Hs4 x Hs4



e) Hc4 x Hc4 ; Hc13 x Hc13



f) Lx19 x Lx19 ; Lx22 x Lx22



relatively slight effects on growth of different *Hebeloma* isolates but more drastically reduced the growth of Laccaria isolates.

All of the *Laccaria* isolates had only slight effects, if any, on the growth of *Hebeloma* isolates, or on each other, but were inhibited to at least some degree, and often markedly, by the presence of *Hebeloma* isolates. Overall, therefore, the results of this experiment support and extend the results for *Hebeloma.Laccaria* interactions in the previous experiment; the presence of *Hebeloma* spp. led to growth- inhibition of *Laccaria* spp., but not vice-versa. Moreover, some isolates of *Hebeloma*, particularly *H.sacchariolens* 6, were effective in reducing the growth of other *Hebeloma* isolates, and the most inhibitory isolates were, themselves, often least affected by others.

5.2.4. The effect of varying strengths of media on the growth of *Hebeloma* spp. and *Laccaria proxima*

In this small experiment, isolates of *Hebeloma crustuliniforme* 4 and *H.sacchariolens* 4 were paired against *Laccaria proxima* 19 on a range of media of different concentrations (PDA at full,half,quarter and one-tenth strength,and Malt Extract Agar and MMN Agar each at full,quarter and one-tenth strength). The plates were incubated at 20°C for 8 wk, there being three replicate plates for each combination. As shown in the table below, evidence of inhibition of *L.proxima* by the *Hebeloma* spp. was seen on most types of medium, even those of low nutrient content.

Summary of the growth of *H.crustuliniforme* and *H.sacchariolens* on three agar types at various strengths.

Agar type	Visible patterns of inhibition	Colony appearance	Evidence of staling
PDA	n.a.	small,dense	+
PDA/2	+	"normal"	o
PDA/4	+	sparse	o
PDA/10	n.a.	sparse	o
MA	n.a.	small,dense	+
MA/4	n.a.	small,dense	+
MA/10	+	sparse	o
MMN	+	"normal"	o
MMN/4	+	sparse	o
MMN/10	n.a.	sparse	o

(n.a. = not applicable because colonies did not grow together sufficiently to show patterns of inhibition or else growth was too sparse to observe colony margins).

However, at the lowest nutrient levels the mycelia were so sparse that interactions could not be recorded, and at the highest nutrient contents both *L.proxima* and the *Hebeloma* spp. showed evidence of staling, which precluded assessments of growth-inhibiting interactions. Colonies showing evidence of staling were extremely small, with crenated margins, and they were associated with brown discoloration of the surrounding agar. This experiment thus showed that the inhibition of growth of *Laccaria proxima* when in close proximity to colonies of *Hebeloma* spp. was repeatable and occurred on at least three types of medium.

5.2.5. Test for the presence of volatile inhibitors

Plates of half-strength PDA were prepared and inoculated centrally with discs of *Laccaria proxima* isolate 19 or *Hebeloma sacchariolens* isolate 4.

After 2 days' incubation at 20 °C, the lids were removed and, in aseptic conditions, the bases were inverted over each other or over uninoculated agar plates in various combinations, with five replicates per combination. The joints were sealed with strips of "Nescofilm". The dishes were then incubated at 20°C for 8 wk, measurements of colony growth being made at intervals.

As shown in the table below, there was no evidence that growth by *L.proxima* was inhibited by volatile metabolites of itself or of *H.sacchariolens*.

Mean radius (mm) of colonies of *L.proxima* on PDA in the presence of colonies of either *L.proxima*, *H.sacchariolens* or no fungus.

Day	*L.proxima*	*H.sacchariolens*	no fungus
14	2.0	2.0	1.9
28	2.7	2.9	2.8
42	3.9	4.1	4.0
56	4.2	4.2	4.2

5.2.6. Interactions between isolates of *Hebeloma* spp. and *Laccaria proxima* in liquid culture

Potato dextrose liquid medium (half strength) was prepared (Section 2.1.2) and dispensed in 50 ml aliquots to 100 ml conical flasks, which were plugged with cotton wool, capped with aluminium foil and autoclaved at 121°C for 15 min.

When cool, each flask was inoculated such that seven isolates of *Hebeloma* spp.(*H.crustuliniforme* 4,8,and 13, *H.sacchariolens* 4,*H.subsaponaceum* 2 and 3 and *Hebeloma* sp.3) were paired with themselves or with *L.proxima* 19, and *L.proxima* also was paired with itself. One disc of agar in each pair was marked by placing a short piece of autoclaved capillary tubing into it. The flasks were

Table 5.2.2

Mean dry weights (mg) of isolates of *Laccaria* and *Hebeloma* grown together in liquid culture (mean of 6 replicates) and dried at 80°C.

Combination	Mean dry weight (+/- s.e.)
Hs4 x Hs4	382 (+/- 53)
Hc4 x Hc4	273 (+/- 39)
Hc8 x Hc8	480 (+/- 74)
Hcl3 x Hcl3	323 (+/- 54)
Hsb2 x Hsb2	260 (+/- 22)
Hsb3 x Hsb3	244 (+/- 25)
Hsp3 x Hsp3	361 (+/- 43)
Lpx19 x Lpx19	507 (+/- 45)

Combination	Mean dry weight HEBELOMA	Mean dry weight LACCARIA
Hs4 x Lpx19	127 (+/- 45)	1039 (+/- 79)
Hc4 x Lpx19	208 (+/- 13)	1179 (+/- 99)
Hc8 x Lpx19	236 (+/- 42)	1154 (+/- 173)
Hcl3 x Lpx19	229 (+/- 23)	1093 (+/- 92)
Hsb2 x Lpx19	173 (+/- 49)	583 (+/- 81)
Hsb3 x Lpx19	179 (+/- 61)	968 (+/- 230)
Hsp3 x Lpx19	194 (+/- 13)	1065 (+/- 145)

incubated, unshaken, at 20°C for 10 wk, then the contents of each flask were emptied into a clean petri dish. The colonies could easily be separated using fine forceps and the glass rods were removed before each colony was placed on pre-weighed filter paper, oven dried at 80°C and its dry weight determined (Table 5.2.2).

Two main trends are apparent in Table 5.2.2. The growth of all isolates of Hebeloma was markedly reduced in the presence of *L.proxima* compared with in the presence of a sister colony. Conversely, the growth of *L.proxima* was increased usually two-fold in the presence of all isolates of *Hebeloma*, with the exception of *H.subsaponaceum* 2.

These results contradict the trends observed in linear growth on agar plates, in which extension of colonies of *Laccaria* was markedly reduced when in close proximity to colonies of some isolates of *Hebeloma*.

5.2.7. Discussion

Interactions between fungi in soil are an inevitable consequence of proximity or contact between individuals of the same of different species (Rayner & Webster, 1984) and on a root system the replacement of one fungal type by another may result from the inhibition of growth of one species by the other and consequential overgrowth (Frankland, 1981).

In this series of experiments, inhibition of growth of *Laccaria* spp. by *Hebeloma* spp. was apparent and repeatable on several agar types, and between some isolates (e.g. *H.sacchariolens* 6 x *L.proxima* 19) the degree of inhibition was marked. However, as with the other experiments involving assessment by radial growth, the extent of hyphal branching was not taken into account; when the

combinations of fungi were tested in liquid culture and biomass production by each species was calculated, the results were the opposite to those recorded on agar, in that growth of *Laccaria* spp. was apparently stimulated by the presence of *Hebeloma* spp. Clearly no reliable evidence for inhibition of one species by another has thus been obtained, and it must be questioned whether reliable information relating to interactions on root systems can be obtained in artificial culture conditions involving exogenously supplied nutrients.

An attempt was made to overcome this problem by establishing mycorrhizas of *Laccaria* spp. and *Hebeloma* spp. on birch seedlings and then juxtaposing the root systems to study possible transfer of mycorrhizal types from established sources of infection. Unfortunately, however, the seedlings did not become mycorrhizal to an equivalent extent by the different mycorrhizal fungi, and it was considered that the initial differences in mycorrhizal establishment would engender major interpretational difficulties, so this line of investigation was discontinued.

5.3. Effects of osmotic potential on growth of mycorrhizal fungi in vitro.

5.3.1. Introduction

Reid (1979) reported that the survival and growth of mycorrhizal trees in dry habitats was greater than that of non-mycorrhizal trees. Read & Malibari (1978) and Duddridge *et al.* (1980) demonstrated the efficiency of hyphae and especially of mycelial strands in the uptake and transport of water over ecologically significant distances, such that mycorrhizal plants could survive dry environments in the immediate vicinity of the roots whereas non-mycorrhizal plants died in similar conditions.

The tolerance of fungi, in general, to water stress and the growth responses of mycorrhizal species to variations in soil moisture tends to differ between species (Griffin, 1972). That such differences may be relevant to the establishment of mycorrhizas was indicated by the study of Worley & Hacskaylo (1959) who showed how a decrease in soil water led to a decrease in overall mycorrhizal formation on *Pinus virginiana* although a black mycorrhizal type increased in abundance as water availability was reduced.

Deans (1979) found that, for uniformly distributed trees, the soil within 1.2 m of the tree base tended to become more dry as the growing season advanced than did soil 1.8 - 3m from the trees. Following from such studies, and in particular the work of Mexal & Reid (1973) on effects of different osmotic potentials on the growth of mycorrhizal fungi in vitro, an attempt was made to see if there are any consistent differences in osmotic tolerance between mycorrhizal fungi from different stages in the reported successions on birch (Ford *et al*,1980; Mason *et al*, 1984; Last *et al*, 1984 a,b,1985).

5.3.2. Growth of mycorrhizal fungi on agar adjusted to a range of osmotic potentials with two osmotica.

Potato dextrose agar plates were prepared with the addition of either mannitol or KCl to obtain a range of osmotic potentials as listed in the table below. The quantities of solutes and the resulting osmotic potentials were calculated from freezing point depression values of mannitol and KCl and the following equation (Laing, 1967).

Osmotic potential = $-2\ RT\ m\ \emptyset$

where R and T are standard coefficients, m is the number of moles of

solute per 1000g solvent and ϕ is the osmotic coefficient at freezing point. It was assumed that PDA without added osmoticum had an OP of -3.4×10^5Pa (Mexal and Reid,1973). Preliminary attempts to use polyethylene glycol as osmoticum were abandoned as the agar medium failed to gel.

Molar concentration of mannitol	OP ($\times 10^5$Pa)
0.05	-6
0.11	-9
0.17	-12
0.25	-16
0.31	-18

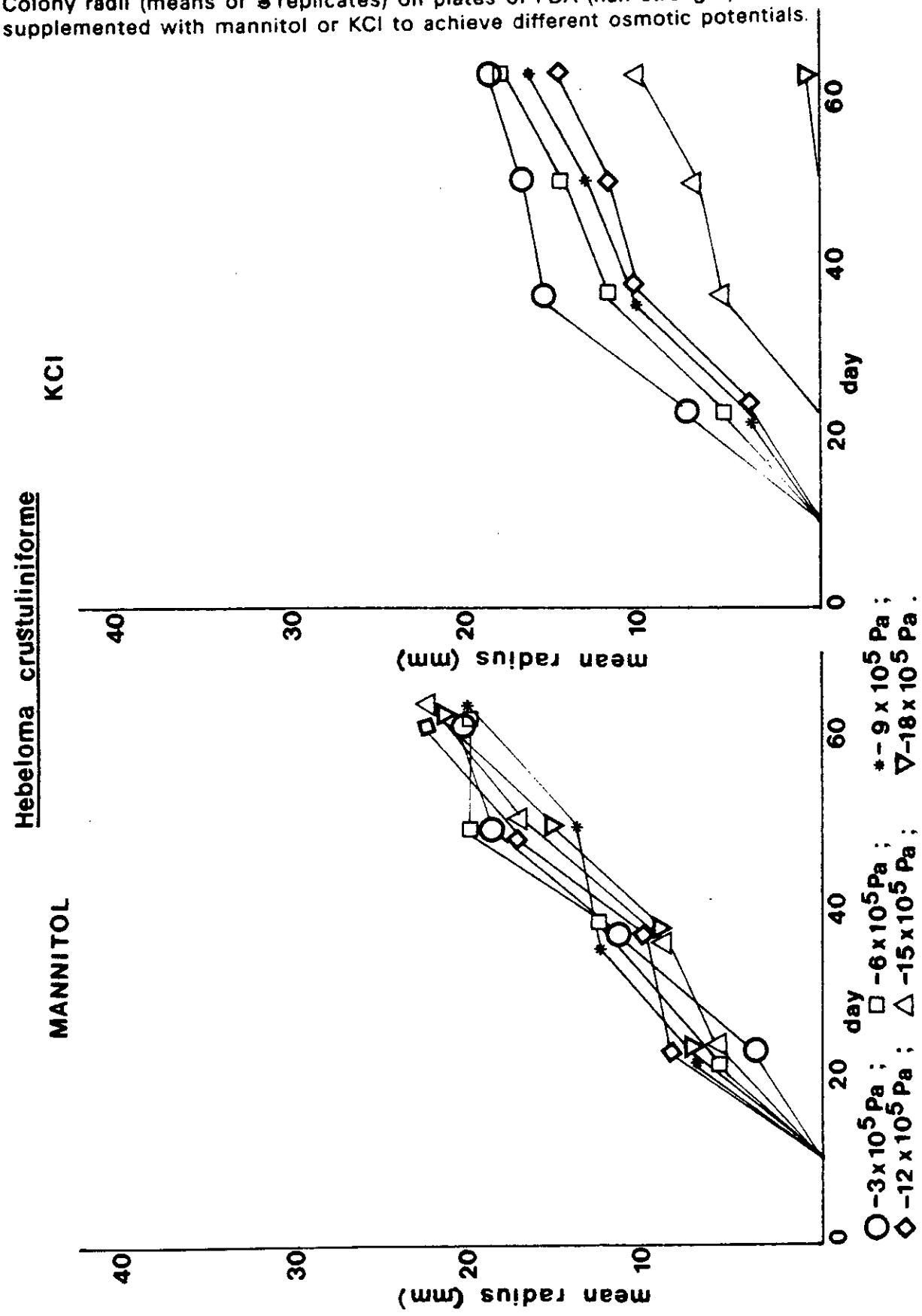
Molar concentration of KCl	OP ($\times 10^5$Pa)
0.01	-6
0.13	-9
0.20	-12
0.27	-15
0.34	-18

Three replicate plates of each agar were inoculated with 6 mm diameter discs of agar taken from the edges of colonies of *Hebeloma crustuliniforme* 4, *H.sacchariolens* 4, *Lactarius pubescens* 4, *Leccinum scabrum* 1, *Paxillus involutus* 16 and *Tricholoma fulvum* 4 that were growing actively on plates of half-strength PDA.

The plates were sealed with cling film and incubated at 20°C for 64 days. During this time, linear growth of the colonies was measured on seven occasions along four perpendicular radii on each plate. Mean growth rates are shown in Figs 5.3.1 - 5.3.6.

All six fungi grew on the basal medium of OP -3×10^5Pa. Relative to this, growth was either unaffected or was reduced by supplementation with mannitol, the only exception being with *H.sacchariolens*, the growth of which was enhanced by mannitol at almost all levels and particularly at OP -9×10^5Pa with

Figs 53.1 - 5.3.6
Colony radii (means of 3 replicates) on plates of PDA (half strength) supplemented with mannitol or KCl to achieve different osmotic potentials.



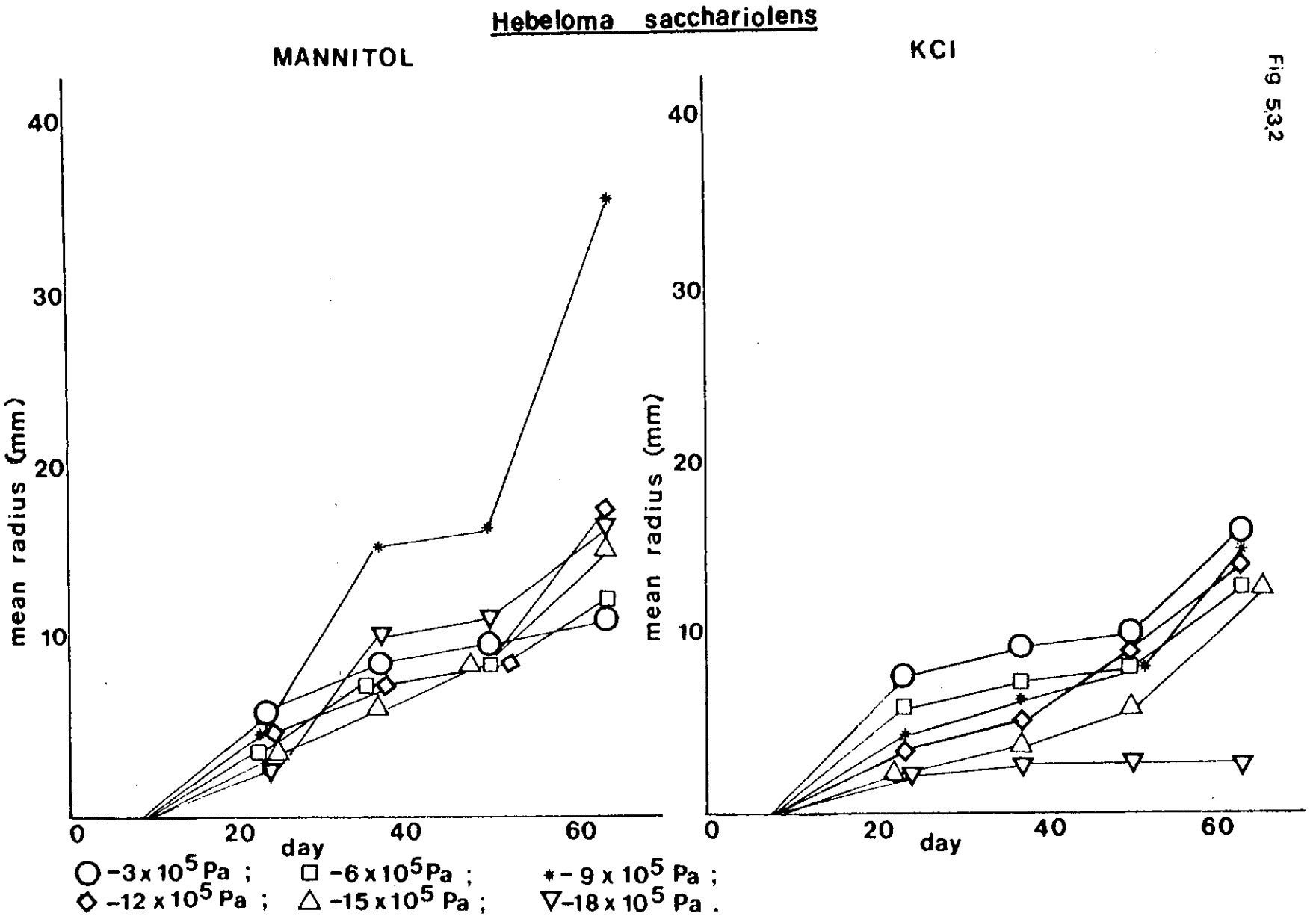

Hebeloma sacchariolens
MANNITOL
KCl
mean radius (mm)
40
30
20
10
0
20
40
60
day
○ -3 x 10^5 Pa ; □ -6 x 10^5 Pa ; * - 9 x 10^5 Pa ;
◇ -12 x 10^5 Pa ; △ -15 x 10^5 Pa ; ▽ -18 x 10^5 Pa .

Fig 5.3.2

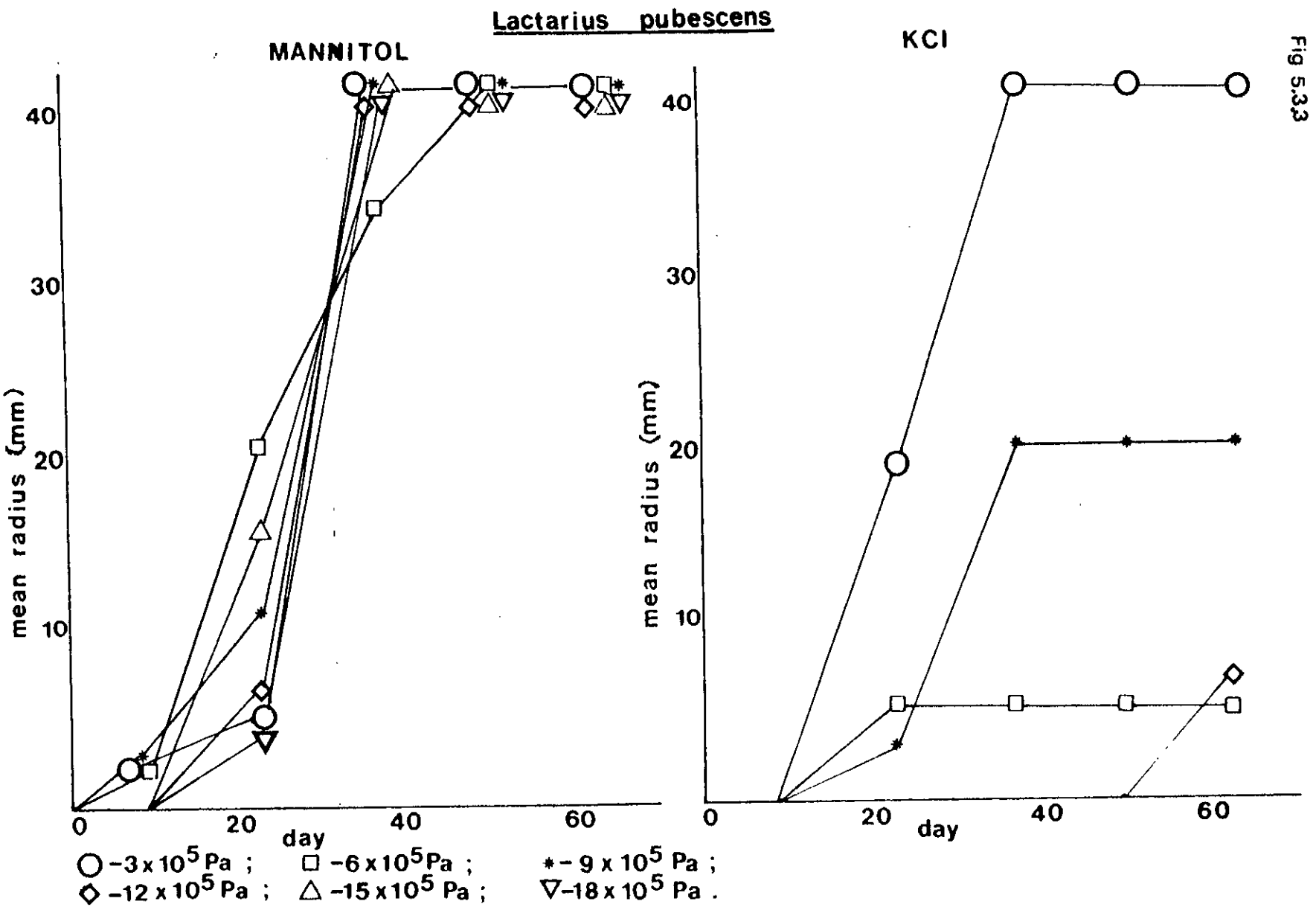

Lactarius pubescens
MANNITOL
KCl
mean radius (mm)
40
30
20
10
0
20
40
60
day
○ -3 x 10^5 Pa ; □ -6 x 10^5 Pa ; * - 9 x 10^5 Pa ;
◇ -12 x 10^5 Pa ; △ -15 x 10^5 Pa ; ▽ -18 x 10^5 Pa .

Fig 5.33

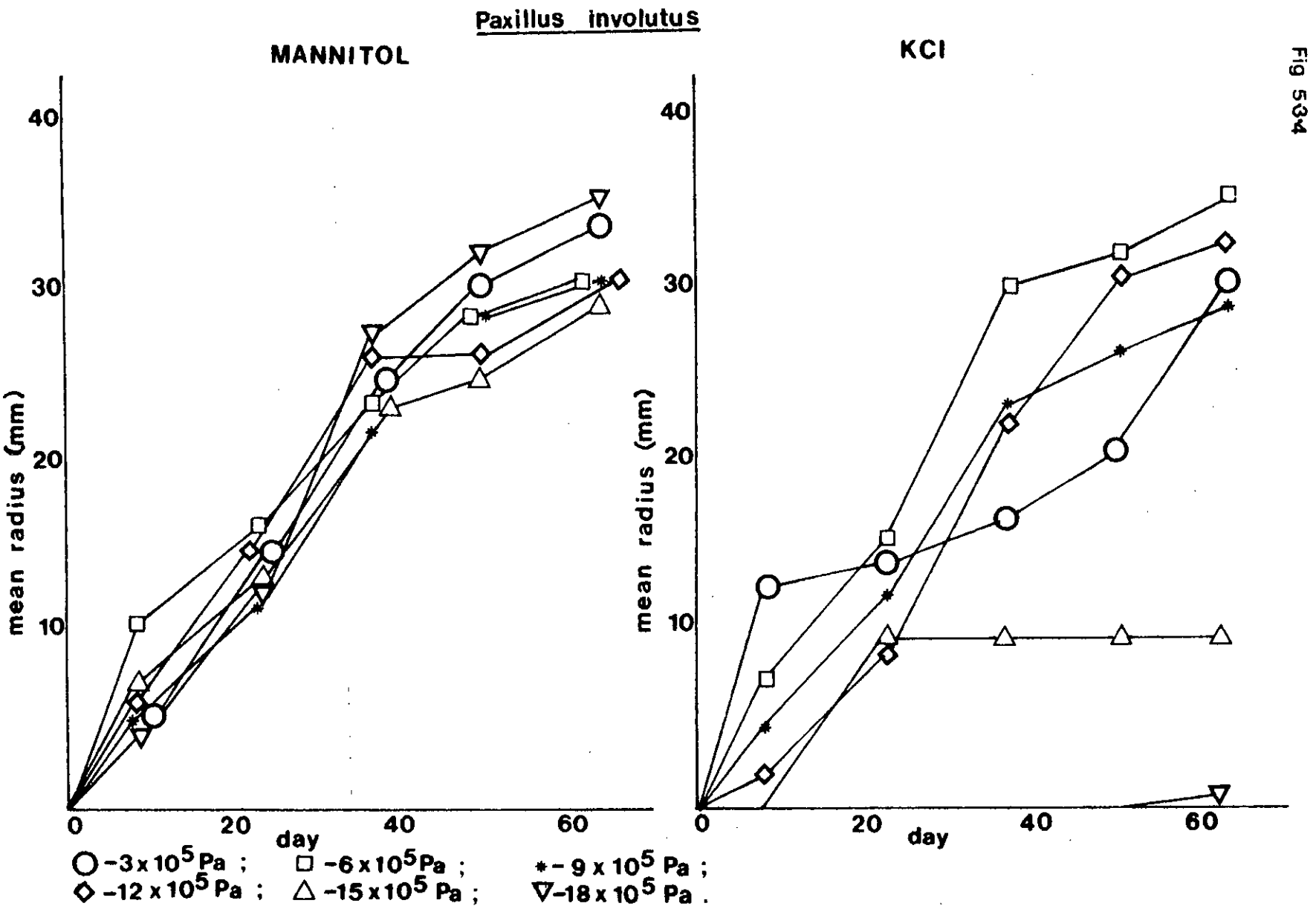

Paxillus involutus
MANNITOL
KCl
mean radius (mm)
40
30
20
10
0
20
40
60
day
○ -3 x 10^5 Pa ; □ -6 x 10^5 Pa ; * - 9 x 10^5 Pa ;
◇ -12 x 10^5 Pa ; △ -15 x 10^5 Pa ; ▽ -18 x 10^5 Pa .

Fig 5-4

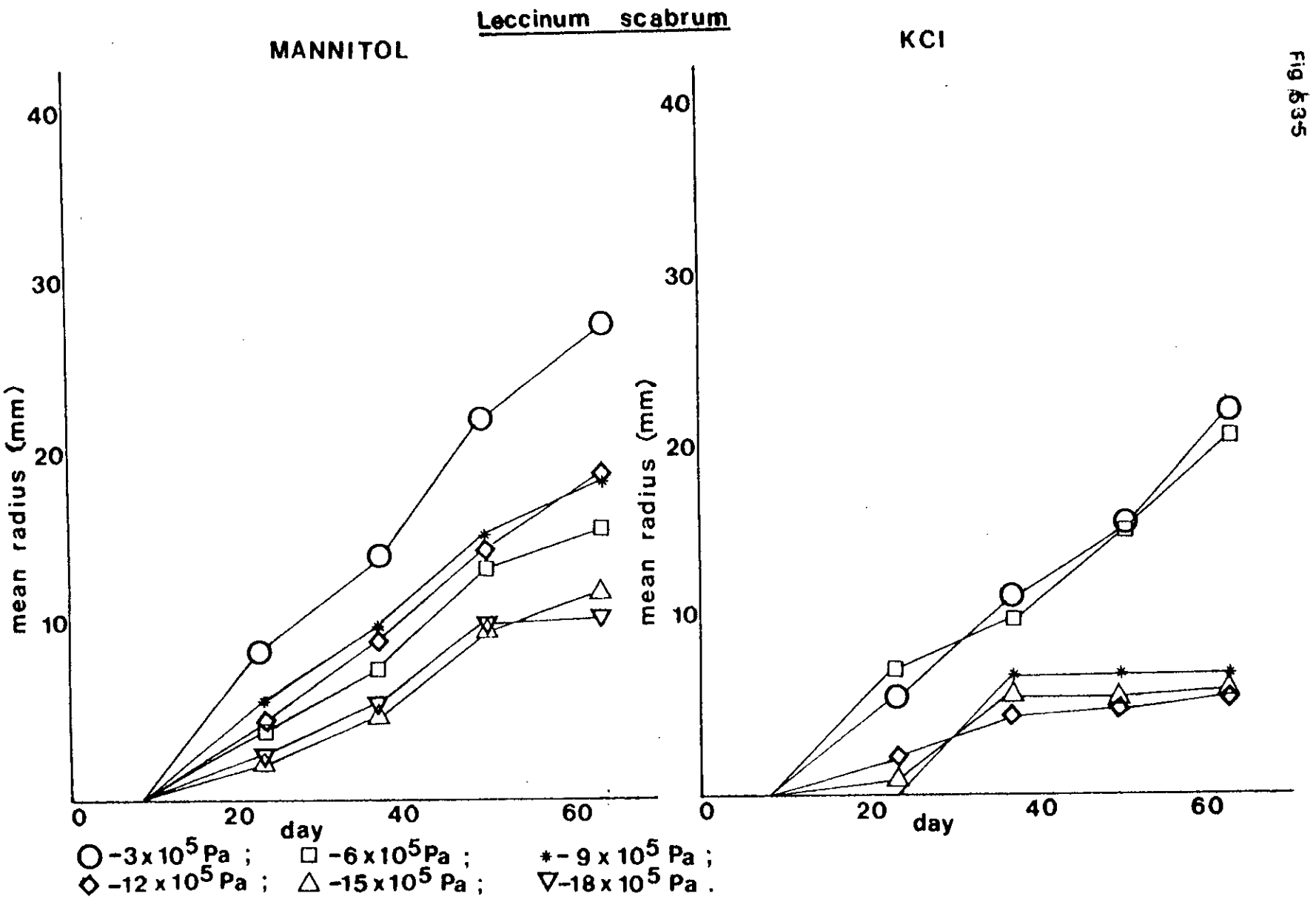
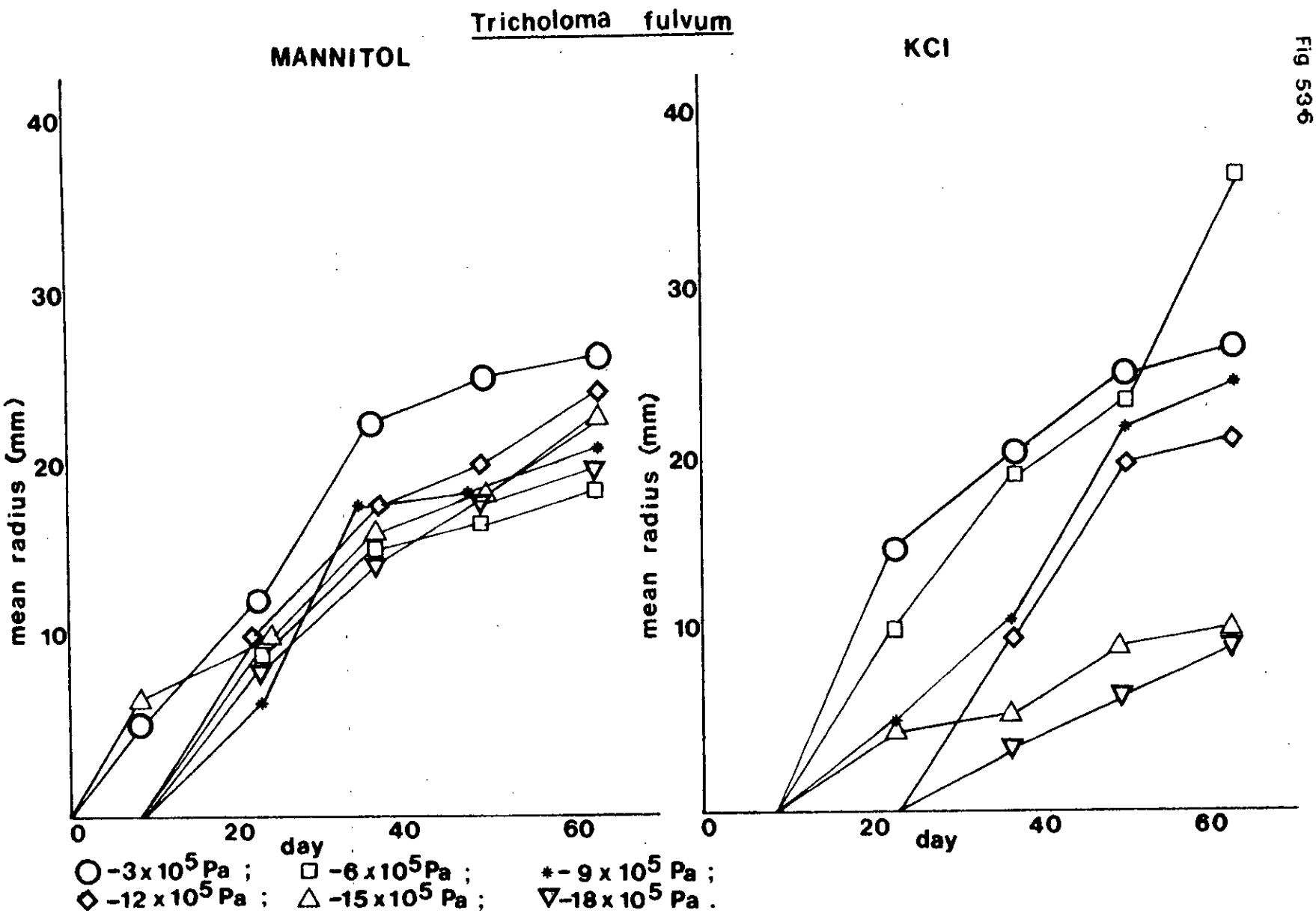

Leccinum scabrum
MANNITOL
KCl
mean radius (mm)
40
30
20
10
0
20
40
60
day
○ -3 x 10^5 Pa ; □ -6 x 10^5 Pa ; * - 9 x 10^5 Pa ;
◇ -12 x 10^5 Pa ; △ -15 x 10^5 Pa ; ▽ -18 x 10^5 Pa .

Fig 6.35

Fig 536


Tricholoma fulvum
MANNITOL
KCl
mean radius (mm)
40
30
20
10
0
20
40
60
day
○ -3 x 10^5 Pa ; □ -6 x 10^5 Pa ; * - 9 x 10^5 Pa ;
◇ -12 x 10^5 Pa ; △ -15 x 10^5 Pa ; ▽ -18 x 10^5 Pa .

increasing time of incubation (Fig 5.3.2). The growth reductions that occurred in the presence of mannitol - for example with *L.scabrum* and *T.fulvum* (Figs 5.3.5 and 5.3.6) - were apparently not dependent on mannitol concentration, but in the case of *L.pubescens* there was a marked growth reduction only at the highest concentration of mannitol (Fig 5.3.3).

In general, supplementation with KCl had a much more marked effect on colony extension than did mannitol, and the effect of KCl was usually concentration-dependent. For example, colony growth of *H.crustuliniforme*, *H.sacchariolens*, *L.pubescens* and *T.fulvum* was very slow or altogether inhibited by KCl concentrations that resulted in an OP of -18×10^5Pa (Figs 5.3.1, 5.3.2, 5.3.3 and 5.3.6), and the next most inhibitory KCl concentration was that resulting in -15×10^5Pa. Only *H.crustuliniforme*, *L.scabrum* and *T.fulvum* remained unaffected by even the lowest concentrations of KCl, while the growth of *P.involutus* tended to be increased at this concentration. In contrast, *L.pubescens* was extremely sensitive to the salt, in marked contrast to the lack of sensitivity of this fungus to any but the highest concentration of mannitol (Fig 5.3.3). Such growth as was made by *L.pubescens* in the presence of low concentrations of KCl was extremely sparse, in contrast to that on unmodified agar or agars supplemented with mannitol.

5.3.3. Growth of mycorrhizal fungi in liquid medium altered to a range of OP with PEG4000

In this experiment, MMN liquid medium (Section 2.1.2) was prepared and supplemented with different concentrations of polyethelene glycol 4000 (Mexal and Reid,1973) to achieve osmotic potentials as follows

% PEG 4000 (w/v)	Final OP (x 10^5Pa)
0	- 1.6
19.5	-10.8
25.0	-21.8
28.5	-30.0

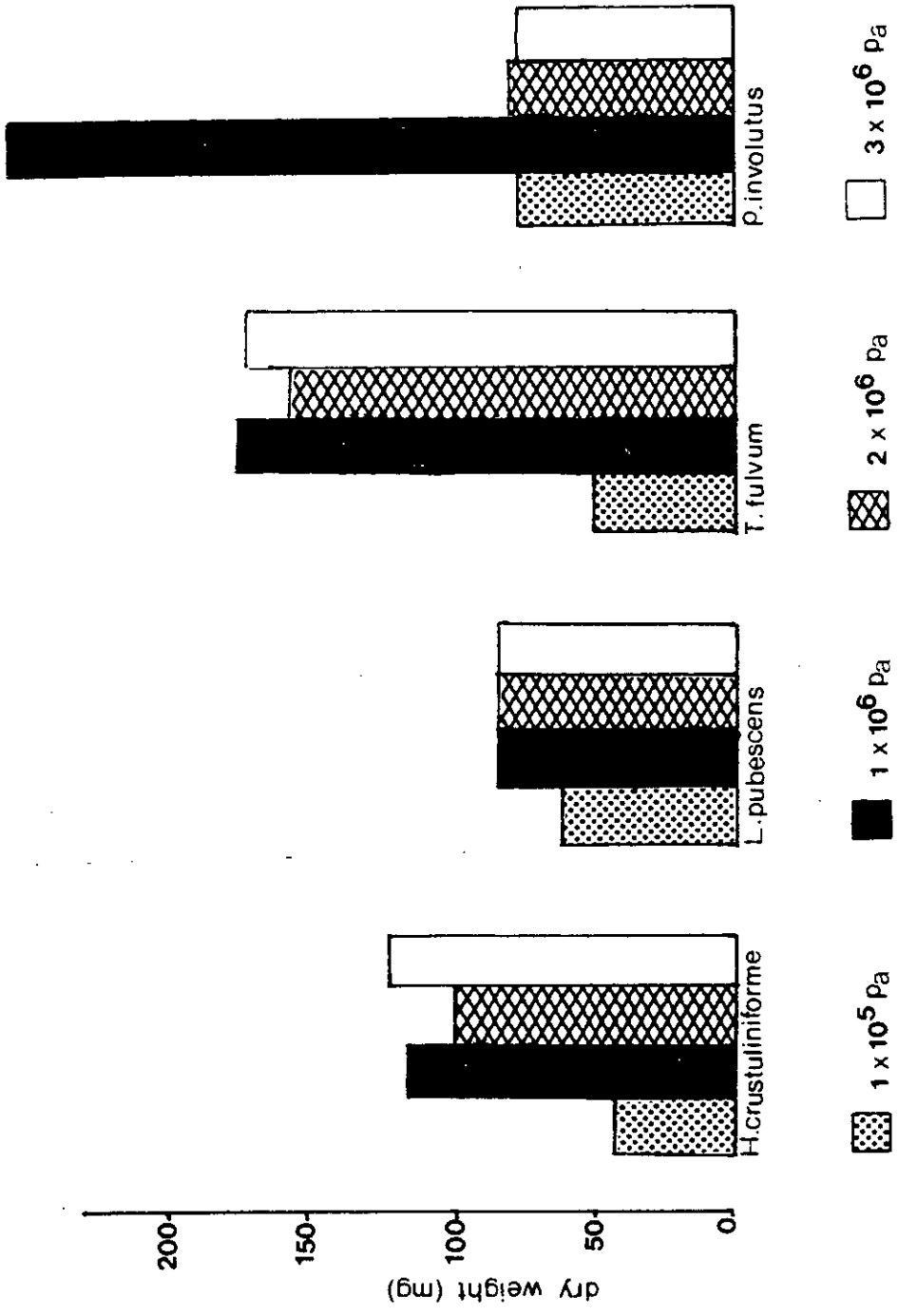
Aliquots (50 ml) of each solution were added to 100 ml conical flasks, together with two glass beads (0.8 mm diameter) to assist subsequent agitation. The flasks were plugged with cotton wool, capped with aluminium foil and autoclaved at 121°C for 15 minutes. They were inoculated with two 6 mm diameter agar discs cut from the margins of colonies of *H.crustuliniforme* 4, *L.pubescens* 4, *T.fulvum* 4 and *P.involutus* 16 on MMN agar. The flasks were incubated at 20°C for 15 wk, being shaken weekly. Then the contents were washed through pre-weighed filter papers in a Büchner funnel, oven-dried at 80°C and weighed (Fig 5.3.7).

Three of the four fungi showed significant increases in mycelial dry weight on addition of the osmoticum : growth was significantly enhanced at -1x 10^6Pa compared with -1 x 10^5Pa, and, compared with the controls, there was no inhibitory effect of the osmoticum at up to -3 x 10^6Pa, the highest level tested. Growth of the fourth fungus, *P.involutus*, again was significantly increased by the addition of PEG 4000 to -1 x 10^6Pa OP, but was unaffected at -2 and -3 x 10^6Pa OP.

5.3.4. Discussion

The colony extension of the isolates on agar tended to be unaffected or was reduced by increasing amounts of the osmotica mannitol or KCl in the medium, the extent of the growth reduction being more marked with KCl. The disadvantages of using mannitol or KCl as osmotica rest on the possibility that

FIG 5.3.7
Growth of four mycorrhizal fungi (as measured by dry weight) in liquid Potato Dextrose medium altered to a range of osmotic potentials with PEG 4000.



these substances may be, respectively, metabolised by or directly inhibitory to the growth of different species of mycorrhizal fungi, thus masking the true effects of increasing OP on their growth. In addition the method of assessment - measurement of colony radial growth - did not take account of differences in density of the colonies.

Growth of the four species tested in liquid medium was influenced by PEG 4000 - a substance reported to be neither directly stimulatory nor inhibitory to fungal growth (Mexal & Reid, 1973) and thus its effects may have been directly attributable to adjustment of OP. In this experiment all four species of mycorrhizal fungi grew better in media amended with the osmoticum than on the unamended medium.

There was no evidence, however, that the late stage species, such as *L.pubescens* and *L.scabrum*, were affected differently from early stage species such as *H.crustuliniforme* and *H.sacchariolens*.

5.4. Genotypic determinants of mycorrhizal formation.

5.4.1. Introduction

Last,Mason,Pelham and Ingleby (1984) recorded the seasonal occurrence of fruitbodies of ectomycorrhizal fungi beneath clones of *Betula pubescens* and *B.pendula* that had been planted as saplings on a previously agricultural site (Farfield) near Edinburgh. Two clones of *B.pubescens* (9.3D and 9.3G) had been raised from cuttings taken from single trees that were originally part of one seedlot from a tree in Crathie, Grampian region, in 1974. They had been grown in two soils - from Field OS 127 and from Farfield (both on the Bush Estate, Midlothian) before planting into the experimental site. These two clones showed significant differences in fungal fruitbodies associated with them: clone 9.3D had a mean 3.3 fruitbodies of *Inocybe petiginosa* and 0.3 fruitbodies of *Laccaria tortilis* in 1978 whereas clone 9.3G had a mean 0.5 and 15.5 fruitbodies of these respective types. Since 1978 no fruitbodies of *I.petiginosa* have been observed, but those of *L.tortilis* were still appearing in 1986.

5.4.2. Method

Cuttings were taken from trees of clones 9.3D and 9.3G in July 1985 and were rooted as described in Section 2.2.3. Later in 1985, fruitbodies of *L.tortilis* were collected from the field site, and fruitbodies of *I.petiginosa* were collected from beneath saplings of Sitka spruce growing in pots in glasshouse conditions. The fruitbodies were chopped finely and shaken in sterile tap water for 5 min. The spore suspension thus obtained were decanted and the spore densities were determined using a haemacytometer. The suspensions were diluted to 42 ml with sterile tap water and aliquots of 1 ml were applied by

syringe just beneath the surface of the soil of the potted cuttings. Seven cuttings of each clone were inoculated with each spore suspension and seven of each were left uninoculated as controls. The plants were maintained in a glasshouse for 16 wk and watered as neccessary. The roots were then washed free of soil under gently running tap water and arranged linearly on a grid. Sections of the root system, one cm wide, were cut with a razor blade, then pooled for each replicate seedling and examined microscopically. The total number of root tips and the proportions of these attributable to different mycorrhizal types were recorded. Shoot heights also were measured.

5.4.3. Results

As shown in Tables 5.4.1 - 5.4.3, there was no significant difference in shoot height, total number of root tips or overall percentage of mycorrhizal infection between the clones or between inoculation treatments. Five mycorrhizal types were identified on the root systems, Thelephora-type being the most common type followed by Inocybe-type, and there were relatively few mycorrhizas of *Laccaria* ,*Hebeloma* and *Cenococcum* (Tables 5.4.4 - 5.4.8).

No significant effect of inoculation with spores of *I.petiginosa* or *L.tortilis* was seen in the experiment, (Tables 5.4.4 and 5.4.5), except that the addition of spores of *I.petiginosa* apparently favoured the development of Thelephora-type mycorrhizas (Table 5.4.6). Similarly, there was little overall difference in mycorrhizal development on the different clones, except that Thelephora-type mycorrhizas were significantly (P=0.05) more common on clone 9.3D than 9.3G., whereas Laccaria-type mycorrhizas were significantly (P=0.05) more common on clone 9.3G that 9.3D (Table 5.4.5). In both of these instances

Table 5.4.1
Shoot height (mm)

Clone	Spore application Ip	Lt	Control	Row mean	SED	5% LSD
9.3D	349	343	395	362	41.2	-
9.3G	450	455	306	403		
Column mean	399	399	350			

SED (50.4)
5%LSD -

Table 5.4.2
Mean number of root tips per plant

Clone	Spore application Ip	Lt	Control	Row mean	SED	5% LSD
9.3D	811	521	757	657	56.1	114.6
9.3G	754	620	597	673		
Column mean	745	571	680			

SED (68.8)
5%LSD -

Table 5.4.3
Mean percentage of mycorrhizal root tips per plant

	Spore application					
Clone	Ip	Lt	Control	Row mean	SED	5% LSD
9.3D	68.8	73.0	76.6	66.7	4.4	-
9.3G	69.4	63.2	67.5	72.8		
Column mean	69.1	68.1	72.0			
	SED (5.4)					
	5%LSD -					

Table 5.4.4
Percentage of root tips attributable to Inocybe

	Spore application					
Clone	Ip	Lt	Control	Row mean	SED	5% LSD
9.3D	10.5	23.0	25.9	19.8	5.1	10.4
9.3G	21.3	25.0	27.8	24.7		
Column mean	15.9	24.0	26.9			
	SED (6.3)					
	5%LSD -					

Table 5.4.5
Percentage of root tips attributable to Laccaria

Clone	Spore application Ip	Lt	Control	Row mean	SED	5% LSD
9.3D	2.7	3.9	3.2	3.2	2.0	4.1
9.3G	4.6	7.5	10.9	7.5		
Column means	3.4	5.7	7.0			

SED (2.4)
5%LSD -

Table 5.4.6
Percentage of root tips attributable to Thelephora

Clone	Spore application Ip	Lt	Control	Row mean	SED	5% LSD
9.3D	53.0	42.9	41.2	45.7	4.4	8.9
9.3G	40.7	27.5	25.7	31.3		
Column mean	46.8	35.2	33.4			

SED (5.4)
5%LSD (10.9)

Table 5.4.7
Percentage of root tips attributable to Hebeloma

Clone	Spore application Ip	Lt	Control	Row mean	SED	5% LSD
9.3D	1.3	2.4	4.9	2.9	1.7	-
9.3G	1.8	1.0	2.2	1.7		
Column mean	1.5	1.7	3.6			
	SED (2.1)					
	5%LSD -					

Table 5.4.8
Percentage of root tips attributable to Cenococcum

Clone	Spore application Ip	Lt	Control	Row mean	SED	5% LSD
9.3D	2.0	0.8	1.5	1.4	0.9	-
9.3G	1.6	2.1	0	1.2		
Column mean	1.8	1.5	0.7			
	SED (1.1)					
	5%LSD -					

Table 5.4.9
Percentage of moribund root tips

Clone	Spore application Ip	Lt	Control	Row mean	SED	5% LSD
9.3D	7.1	4.1	5.5	6.2	2.3	-
9.3G	9.6	7.1	1.9	5.5		
Column mean	8.3	5.6	3.7			

SED (2.8)
5%LSD (5.7)

the effect was consistent across the inoculation treatments. The result for *Laccaria* is of interest because it is consistent with the report by Last *et al.* (1984 a) that clone 9.3G supported more fruiting by *L.tortilis* than did clone 9.3D.

Hebeloma and *Cenococcum* (Tables 5.4.7 and 5.4.8) occurred on 20 and 11 plants respectively, accounting for up to 5% of the root tips. Spore application treatments had no significant effect on the formation of mycorrhizas of either of these types but both were significantly (P=0.05) more frequent on clone 9.3D than 9.3G.

Moribund root tips (Table 5.4.9) were observed on 32 of the 42 plants. Although they were significantly (P=0.05) more common on plants inoculated with *l.petiginosa* than on uninoculated plants, they occurred to a similar extent on both clones.

5.4.4. Discussion

Some mycorrhizal fungi have broad host selectivity. *Cenococcum graniforme*, for example, has been observed in association with birch,pine,larch,beech and oak (Mikola, 1948). In contrast, some other mycorrhizal fungi tend to be found almost exclusively in association with particular types of tree - for example Suillus grevillei in association with larch and some pines. The host may also exhibit a degree of specificity or selectivity in terms of the types and number of types of fungi with which it forms mycorrhizas. Wright & Ching (1962) observed differences in the numbers of mycorrhizal associates of Douglas fir seedlings from different provenances and Marx & Bryan (1975) found that even half-sib progenies of slash pine varied in their degree of asociation with *Pisolithus tinctorius*.

Last *et al.* (1984 a) reported variation in the mycorrhizal species that formed fruitbodies around clones of *B.pubescens*. The results of my experiment, which involved inoculation of cuttings taken from the trees observed by Last *et al.* (1984 a) did not entirely confirm the results of these workers, although the experiment did reveal that Laccaria-type mycorrhizas developed better on cuttings from the clone that previously had supported substantial fruiting by *L.tortilis*. It is interesting to note that whereas fruitbodies of *Inocybe petiginosa* have not been observed on either clone for several years, during routine field observations, yet fruitbodies of *Laccaria tortilis* were still observed in 1986.

CHAPTER 6

CONCLUDING DISCUSSION.

Fungal successions in general have been discussed in detail by Park (1968), Frankland (1981) and Cooke & Rayner (1984). Park (1968) made a basic distinction between substratum and seral successions. Substratum succession was considered to represent changes in fungal community structure with time on a substratum or piece of material such as leaf litter or wood. Seral succession was viewed as a procession of changes often concurrent with, or correlated with, changes in biotic and other features of a habitat. An example of a seral succession of fungi, discussed be Frankland (1981), is the change in fungal community structure on a sand dune system, moving progressively from foredunes to established woodland on mature dunes. As pointed out by Frankland, however, such a successional change can often only be inferred by sampling at a single time rather than by following the course of events as they occur.

Seral successions of mycorrhizal fungi seem to occur in nature. A simple example is the change from a predominance or exclusive development of vesicular-arbuscular mycorrhizal fungi on plants growing on foredunes (Daft & Nicolson, 1974) to the eventual predominance of ectomycorrhizal fungi on tree root systems on old stabilised dunes. At some point in such a succession there may also be a third type of mycorrhizal fungus, such as *Rhizoctonia* spp., growing in association with orchids such as *Listera ovata* in stabilised dunes. A similar type of seral successionoccurs in reclaimed land or mining spoil heaps, as herbaceous plants are progressively replaced by woody plants such as birches (Bradshaw & Chadwick, 1980).

There have been numerous reports of observed fruiting of different mycorrhizal types in association with trees of particular ages. Chu-Chou (1979) observed fruitbodies of *Hebeloma* and *Laccaria* spp. in nurseries of *Pinus radiata* but not in outplantings, and fruitbodies of *Amanita muscaria* only in stands over 10 years old. Chu-Chou & Grace (1981) extended this study, in this case on Douglas fir, to examine the mycorrhizas associated with nursery plants and 15 yr old stands and again found *Hebeloma* only on nursery plants and *Amanita* on older trees. A similar pattern of age-dependent associations have been observed in the long term study of fruitbody production in association with birch at ITE, Bush Estate (Ford *et al.*, 1980; Mason *et al.*, 1982. 1983b, 1984; Last *et al.*, 1984a,b). In so far as the habitat changes as these woodlands age this type of age-dependent association - succession - could be considered seral. Alternatively it could be argued that the plant species themselves do not change as the woodlands age; instead the component parts merely age, and as these are the substrate of the ectomycorrhizal fungi the succession is really a substratum succession. Regardless of such arguments, there is accumulating evidence that the community structure of mycorrhizal fungi does change with age of a woodland, and the evidence for this will now be briefly reviewed and reasons for it will be discussed in relation to the work in this thesis.

A working definition of the term "succession" as applied to fungi was provided by Cooke & Rayner (1984) as follows:

> "the sequential occupation of the same site by thalli (normally mycelia) either of different fungi or of the different associations of fungi".

Notable in this respect is the fact that a succession represents a change with time and is not applicable directly to changes in space, but the work to date on mycorrhizal succession has distinguished between spatial and

temporal successions (Ford *et al.* 1980; Mason *et al.* 1982, 1983; Deacon *et al.* 1983; Last *et al.* 1983).

Most evidence for spatial and temporal successions of mycorrhizal fungi has come from studies on experimental birch plots at ITE, Bush Estate, but is supported to some degree by other work on pines (Becker, 1956; Tominaga, 1975), Douglas fir (Chu-Chou & Grace, 1981) and eucalypts (Gardner *et al.* 1985). The evidence is mainly derived from mapping of fungal fruitbodies, the mean positions of which changed as the trees aged (Ford *et al.* 1980; Mason *et al.* 1982; Last *et al.* 1983). The evidence to relate spatial with temporal successions is relatively weak, but it seems reasonable to assume that these are both part of the same phenomenon. Thus, the first fruitbodies to appear in young birch stands were of types that occurred in subsequent years but further from the tree bases than in earlier years; the later fruitbody types that appeared tended to occur closest to the tree bases.

Such a coincidence of spatial and temporal patterns might be explained if the root tips formed on a part of the root system that was of a particular age are susceptible to infection by a particular fungus. In other words, if the root tips on, say, a 2 year-old region of the root system are perhaps especially susceptible to infection by fungus "x" - then this fungus "x" might first be seen on a 2 year-old tree and its position would change in succeeding years by an amount equivalent to one years' radial spread of the root system. Unfortunately, the data from fruitbody mapping at ITE (Ford *et al.* 1980; Mason *et al.* 1982; Last *et al.* 1983) have not been related sufficiently closely to the rates of spread of root systems to test such a hypothesis.

A more serious limitation to the data than that mentioned above is the fact that most of the evidence for mycorrhizal successions on birch was based on the occurrence of fruitbodies. Harper & Webster (1964) showed, for

coprophilous fungi, that the sequential occurrence of reproductive structures can be misleading, because different fungi require different periods of vegetative activity before they produce their reproductive structures. Indeed, Deacon *et al* (1983) recorded that the fruitbodies of the earliest mycorrhizal fungi to fruit on the birch plot at ITE were relatively small, whereas the fruitbodies of the fungi that appear only later in the observed succession are larger and heavier. Mason, Wilson & Last (1984) reported that J.H.Warcup had examined the actual mycorrhizas in a series of positions around birch trees in the experimental plot at ITE and in this "blind" sampling of predetermined positions, had recorded the presence of mycorrhizal types that matched the identity of the fruitbodies that had recently appeared in those positions. Unfortunately, the results of this investigation were not published. In any case, it can be questioned whether the results of such a study are meaningful; the design of the sampling was to examine mycorrhizas in positions in which fruitbodies had recently occurred, but this would be expected to reveal a predominance of mycorrhizas of the fruiting fungus, as shown by Deacon *et al.* (1983). However, there were instances in which mycorrhizas of the fruiting fungus did not predominate (Deacon *et al*, 1983) and this was also the case in one of my experiments (Section 4.4). Moreover the sampling method employed by J.H.Warcup did not reveal whether the same types of mycorrhizas also occurred in equal abundance where fruitbodies were absent. In fact, the best evidence to relate, independently, mycorrhizal abundance and changes in mycorrhizal status with distance around birch trees is that of Deacon *et al.* (1983) who showed that in soil cores taken at 25 cm intervals from tree bases the predominant mycorrhizal types changed with distance. Subsequently, Fleming *et al.* (1984) similarly showed a spatial succession of mycorrhizal types around young (4 yr old) birch trees in a field site on Bush Estate. There is, thus, a limited amount of direct evidence of a spatial

succession of mycorrhizas *per se* on birch, but there is no equivalent direct evidence of a temporal succession as no-one has done this type of sampling in successive years. Limited evidence of a temporal succession has been provided by, among others, Chu-Chou (1979) for pine stands, Trappe & Strand (1969) and Chu-Chou & Grace (1981) for Douglas fir, and by Last *et al* (1981) for birch, in which types of fungal fruitbodies occurring on young saplings were different from those observed in mature stands.

Part of the work in this thesis was aimed at demonstrating the existence of spatial and temporal successions, or sequences, of mycorrhizal fungi in experimental conditions (Chapter 4). Unfortunately, however, the work was rather inconclusive in this respect, possibly owing to the relatively short time (maximum 2.5 yrs) over which sequential observations of mycorrhizas could be made in troughs of soil. Over the course of the experiments, mycorrhizas of proposed late-stage fungi such as *Lactarius* came to predominate on the older parts of birch sapling root systems (Table 4.4.14) but in at least the early stages of the experiment they were observed on the lower soil surface even on the youngest regions of root systems (Fig 4.4.3). Mycorrhizas believed to be of *Leccinum* spp. and *Paxillus* spp. also predominated on the older parts of the root systems in troughs, although these mycorrhizal types were relatively uncommon on the root systems and so few conclusions can be drawn from records of their occurrence. In contrast, sequential observations of the mycorrhizas visible to the naked eye on the lower soil surface revealed no conclusive evidence that mycorrhizas of one type were consistently replaced by those of another type in any part of a root system. Such changes were observed in a few troughs but in most troughs there was a large degree of stability of the patterns of mycorrhizal development over the period of observation (Section 4.4). A further notable point is that there was, in general, little correlation between the positions at which

fruitbodies occurred and the degree of mycorrhizal development in equivalent parts of the root systems (Section 4.4). It seemed instead that the sites at which fruitbodies formed were determined by factors other than , or in addition to, merely the density of development by a particular mycorrhizal type. Two points should be made in this respect.

Firstly, only one fruitbody of *Lactarius pubescens* occurred in the entire 2.5 years' duration of the experiments and yet mycorrhizas of Lactarius-type were relatively common in some troughs and this might have been expected to lead to fruiting of *Lactarius*. Perhaps *Lactarius* requires a long period of vegetative development before fruitbodies are formed, unlike the case with *Thelephora*, *Inocybe* and *Hebeloma* which fruited quite commonly in the troughs. The second point is that fruiting may occur only towards the end of a period of vegetative activity, as is known in some other fungi such as *Schizophyllum commune* (Wessels *et al.* 1979) and when the fungus has sufficient substrate to support fruiting. Such a suggestion was made for ectomycorrhizal fungi by Fleming *et al.* (1986) to explain why mycorrhizas of late-stage species did not develop on seedlings planted into soil cores taken beneath fruitbodies of those late-stage species, despite the presence of abundant inoculum in the cores. This was also found to be the case for late-stage species by Deacon *et al.* (1983). If it is true, then perhaps the failure of *Lactarius* to fruit in the troughs described in Section 4.4 was due to the fact that *Lactarius* had not reached the stage at which its vegetative activity was about to end. Anyhow, it now seems clear that observations of troughs would need to extend over a period longer than 2.5 years in order for good evidence of successional patterns of either fruiting or mycorrhizal development *per se* to be obtained.

The evidence for mycorrhizal successions led to the use of the terms

"early-stage" and "late-stage" to describe the fungi that fruited early of later in the development of the birch stand at ITE. Inevitably this is an artificial distinction because it recognises only two parts of a spectrum (see, for example, Last *et al.* 1985) and Fleming (1985) suggested that *Lactarius pubescens* has features that best fit it to a "middle" category. Nevertheless, the early - late stage distinction seems to have some validity. Repeatedly, experiments have shown that the early-stage species can infect birch seedlings readily from spores or dispersed mycelial inocula in unsterile (non-woodland) soils whereas late-stage species cannot do so (Fox, 1983,1986; Deacon *et al.* 1983). Fleming *et al* (1984) also showed that *Lactarius* spp. could infect birch seedlings planted around mature trees, provided that the soil was not cored or trenched to isolate inoculum of *Lactarius* from contact with the photosynthesizing "parent" tree. More recently, however, Fleming *et al.* (1986) could not confirm this result in a mature coniferous woodland which had been felled leaving stumps. Then seedlings developed mycorrhizas of *Lactarius* when planted round stumps irrespective of coring of the soil.

Part of the work in this thesis was designed to investigate differences in the behaviour of early- and late-stage species of mycorrhizal fungi, both in vitro and in the presence of host roots.

One possibility that was studied was that early- and late-stage species infect at different times in the life of a tree (and at different positions within the root zone of a tree of given age) because the mineral nutritional status of the soil is altered by roots as they explore it. It is known for many mycorrhizal associations that soil phosphorus, and to a lesser degree nitrogen, status influences the success of mycorrhizal development (Lamb & Richards, 1974; Beckjord *et al.* 1980). However, experiments on agar slopes in which the

concentrations of N and P were altered did not reveal consistent effects on mycorrhizal establishment by early- and late-stage species (Section 5.1). Rather, seedlings inoculated with each species (*L.proxima, H.crustuliniforme, L.pubescens* and *A.muscaria*) tended to be smaller and develop fewer mycorrhizas in agar containing one-tenth concentration of phosphorus than in full strength media and seedlings in agar containing one-tenth nitrogen tended to be smallest and to become mycorrhizal only poorly ; these trends were apparent irrespective of inoculum type. Indeed, differences between two isolates of each species of inoculant fungus sometimes were greater than between two different species and the growth of the some species of mycorrhizal fungi on agar in the absence of a seedling followed similar trends.

Another possibility that was investigated concerned the tolerance of different osmotic potentials by mycorrhizal fungi, following the work of Mexal & Reid (1973). Again, however, there were no obvious distinctions between the growth of early- and late-stage species on agar altered to different osmotic potentials and it became clear that this type of study is complicated by the choice of osmoticum used in the experiments. It might be useful to examine the effects of different mineral nutrition status and water potentials on mycorrhizal infection in soil, in which case the soil water potential might best be manipulated by maintaining soils on suction plates to achieve differences in matric potential. However, the preliminary experiments reported here did not give sufficiently promising results to suggest that these lines of investigation should be pursued.

The reported successions on birch roots might be explained if newly-formed root tips on differently aged parts of a root system show differential susceptibility or receptivity to infection by different mycorrhizal fungi. In other words, are young roots on an older region of a root system behaviourly

different (with regard to mycorrhizal infection) from similarly aged root tips on a younger part of a root system ? A critical test of this was devised whereby two birch saplings were planted at opposite ends of troughs of soil such that their root systems overlapped, youngest to oldest regions. Then the root systems were uniformly inoculated with mycorrhizal fungi representing early- and late-stage species (Section 4.3). Unfortunately, there was no evidence that the mycorrhizal fungi established from the introduced inocula in this experiment, and it was also found to be impossible adequately to sample roots of the respective trees. There was some evidence that different trees growing in the same troughs became infected by different mycorrhizal fungi (Fig 4.2.3) but this occurred more or less uniformly along the lengths of the troughs. Tree 1 (Fig 4.2.3) bore mycorrhizas of *Hebeloma* and *Inocybe* all along the roots whereas no Inocybe-type mycorrhizas were observed on Tree 2 even though the roots lay together in close proximity, but this can perhaps be ascribed to genotypic differences between the trees in their susceptibility to infection by different mycorrhizal fungi. (Of interest, *Inocybe* was also apparently unable to infect seedling roots in troughs in which sapling roots, beside which the seedlings were inserted, did bear mycorrhizas of *Inocybe*. This behaviour contrasts with that of *Lactarius* as reported by Fleming (1984) and it merits further study. However, it has not yet been possible to isolate *Inocybe* spp. readily in pure culture so any further work on this fungus would have to involve spore inocula (Fox,1983,1986).)

The phenomenon of genotypic variation with regard to fruitbody production by different mycorrhizal fungi had been previously reported for clones of birch planted into a field site (Farfield) near Bush Estate (Last *et al*, 1984a); of a pair of clones (9.3D and 9.3G) of *Betula pubescens* propagated in the same soil type, one clone (9.3D) supported significantly more fruitbodies of *Inocybe petiginosa* than of *Laccaria tortilis* whereas the reverse was true for clone

9.3G. Thus Last *et al.* (1984a) concluded that production of fruitbodies of - and by extension, the susceptibility to infection by - different mycorrhizal fungi was strongly dependent on host genotype, and my experiment (Section 5.3), involving the inoculation of cuttings from clones 9.3D and 9.3G with spores of *L.tortilis* and *l.petiginosa*, at least partly confirmed these results.

The differences in mycorrhizal development between species and clones of trees may relate to the extent and composition of the root exudates. Smith (1969) examined the exudates from seedlings and 55 year old sugar maple and recorded differences in the proportion of sugars and organic acids in the exudates; Meyer (1966) also observed a difference in the sugars exuded from older and younger parts of the same root system and it is possible that the mycorrhizal successions observed in association with birch are at least partly influenced by the quantity and quality of the root exudates. The experimental results in Section 5.1 and 5.2 of this thesis tend to corroborate this idea in that the late-stage species *Lactarius pubescens*, *Leccinum scabrum* and *Amanita muscaria* tended to form a greater proportion of mycorrhizas with birch seedlings in the presence of higher rather that lower quantities of sugar in agar medium. In addition, mycorrhizas of Lactarius-type were significantly more common on birch sapling roots in troughs (Section 4.4) in regions of the root system close to the tree base - which, according to Meyer (1966) may exude greater quantities of sugars - than at the periphery of the root system. Fleming (1986) suggested that a major difference between early- and late-stage mycorrhizal fungi might relate to the inoculum potential required for establishment of infection - the late-stage species such as *Lactarius* requiring more of a nutrient base in order to infect and thus being favoured by the presence of an older tree as a base from which to infect seedlings.

The last major approach adopted in this thesis was the use of microwave irradiation to simplify the soil microflora and its mycorrhizal components, so that the possible involvement of microbial competition in mycorrhizal development could be studied. Bowen & Theodorou (1979) had previously reported that components of the soil microflora could influence the growth of mycorrhizal fungi on some media and Salt (1970), Suslow & Schroth (1982) and Geels & Schippers (1986) have isolated deliterious rhizosphere bacteria in agricultural soils. An encouraging feature of my work was the demonstration that different mycorrhizal fungi showed differential susceptibility to microwave irradiation, presumably because their propagules exhibit different degrees of heat sensitivity - *Hebeloma*, for example, tended to be eliminated from soil irradiated for 180 sec (reaching approximately 96°C, Section 3.5) whereas *Thelephora* predominated even after 180 sec exposure. This approach to mycorrhizal studies might therefore have value in studies of interspecies anatagonism or competition for establishment of mycorrhizas on seedling roots. However, it was found that the late-stage fungus *Amanita muscaria* could not establish mycorrhizas from added inoculum even if the soil had previously been exposed to microwaves for a substantial time (Section 3.). *Lactarius* did establish mycorrhizas from added inoculum even in non-microwaved soil (accounting for 31 % of root tips)(Section 3.2) but it occurred to a greater degree in soil exposed to microwaves for 80 sec (accounting for 71 % of root tips) ; in another experiment, however, *Lactarius* did not establish infection from added inoculum. These results suggest that simplification of the soil microflora as a result of microwave irradiation can facilitate infection by some late-stage (or "middle"-stage) species, but only in conditions in which those fungi would develop mycorrhizas to at least some degree in the absence of microwave treatment.

In conclusion, my work has not revealed any single factor that can account in large part for the difference in behaviour of early- and late-stage mycorrhizal fungi. Some factors, such as the progressive change in organic matter content of soil or other soil properties with increasing age of a woodland soil, remain to be investigated as differentiating factors in mycorrhizal development and attention might usefully be given to differences in soil properties in future work.

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