ESTABLISHMENT, PERSISTENCE AND SPREAD OF SHEATHING MYCORRHIZAL FUNGI ON ROOTS OF BIRCH (*Betula* spp.)

by

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To My Parents

SUMMARY

Laboratory, glasshouse and field experiments were done to investigate the reasons for a reported succession of sheathing mycorrhizal fungi on birch (*Betula* spp.) and to compare the behaviour of reported 'early-stage' fungi (e.g. *Hebeloma* spp., *Laccaria* spp., *Inocybe* spp. and *Thelephora terrestris*) and 'late-stage' fungi (e.g. *Lactarius pubescens*, *Leccinum* spp. and *Amanita muscaria*) in the succession.

'Early-stage' fungi, in contrast to 'late-stage', readily established mycorrhizas on birch seedlings from either introduced or resident inoculum sources in unsterile soil; in doing so, each fungus tended to exclude others, though the outcome of these interactions was influenced by soil type. 'Early-stage' fungi also persisted and colonised new roots when seedlings on which they had been established in aseptic conditions were transplanted to unsterile soil, whereas only *L. pubescens* of the 'late-stage' fungi persisted on the roots - and then only poorly. Prior establishment of 'early-stage' fungi did not facilitate development of 'late-stage' mycorrhizal types.

Lactarius pubescens and *Leccinum* spp. ('late-stage') established large numbers of mycorrhizas on birch seedlings planted in the root zone of mature birch trees, but only if the seedlings were grown in undisturbed positions - not if the soil was isolated from the parent tree by coring or trenching. Based on this and other evidence, it is concluded that 'late-stage' mycorrhizal fungi characteristically establish on seedlings from mycelial strands or other hyphae that are supplied with a source of photosynthate from parent trees.

It is suggested that 'late-stage mycorrhizal fungi normally require a high inoculum potential for establishment of infection on seedling roots, whereas 'early-stage' fungi can infect from a low inoculum potential.

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DECLARATION

I hereby declare that this thesis has been composed by myself and that all the work herein is my own.

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SECTION I

Introduction

1.1 General Introduction

The term mycorrhiza was first used by Frank (1885) to describe the composite structures formed between some soil fungi and the fine roots of plants in the Cupuliferae and Pinaceae. Later he distinguished two types of mycorrhizas, endotrophic and ectotrophic (Frank, 1887).

In endotrophic mycorrhizas the root cortical cells are penetrated by fungal hyphae, intracellular, without visibly altering root morphology (Sanders, Mosse and Tinker, 1975); they occur on probably 90% of vascular plant species (Trappe, 1977). By contrast, ectotrophic mycorrhizas have a complete external sheath of fungal hyphae, with only intercellular penetration of the root cortex to form the Hartig net. Fungi forming ectotrophic associations include members of the Basidiomyocotina (Trappe, 1962), Ascomycotina (Trappe, 1969) and Endogonaceae in the Zygomycotina (Gerdeman and Trappe, 1974). Their hosts are woody perennials and most commonly are members of the Pinaceae, Salicaceae, Betulaceae and Fagaceae (Meyer, 1973).

Since Frank's discovery (1887), a third type of association, termed ectendotrophic, involving intra- as well as inter-cellular fungal hyphae has been described by Melin (1923). Harley (1969) has suggested that the differences between ectotrophic and endotrophic mycorrhizas may have been overemphasised. Both Rayner (1934) and Clowes (1951), for example, have reported variable amounts of intracellular hyphae in the ectotrophic mycorrhizas of pine and beech, respectively. For this reason, the term "sheathing mycorrhiza" to describe Frank's (1887) original ectotrophic type as suggested by Lewis (1973) is preferred. Hereafter, any future reference to mycorrhizal fungi will mean sheathing mycorrhizal fungi only unless stated otherwise.

1.2 The Symbiotic Association

Mycorrhizal fungi can be classed as ecologically obligate symbiotic biotrophs (Lewis, 1973). As biotrophs, the fungi derive their energy from living host cells; in nature they are dependent on the host for their existence but they may, for example, be cultured in laboratory media, hence they are ecologically obligate symbionts. However, the symbiosis is mutualistic and so the host plant may benefit from the mycobiont.

1.2.1 Fungal Dependence on the Host

The transfer of carbohydrates from a plant to its mycorrhizal fungi was first demonstrated by Melin and Nilsson (1957); labelled carbon compounds produced in photosynthesis were found to accumulate in the fungal cells of the sheath. Lewis and Harley (1965) discovered that these host-derived carbohydrates were converted in the fungal tissues into trehalose, mannitol and glycogen, the authors suggesting that this precluded a return flow to the root. However, as Björkman (1960) and Reid and Woods (1969) have demonstrated that labelled carbon can be transferred from plant to plant via the hyphae of mutually shared mycorrhizal fungi, it is likely that a two-way flow of some carbon compounds is possible.

Most mycorrhizal fungi seem to rely on the products of current photosynthesis, supplied by the host, for the production of their fruitbodies. Rommell (1938) found that the fruitbodies of mycorrhizal fungi were not produced when root-containing plots of soil were isolated from nearby spruce trees. An actively photosynthesising host was shown to be necessary for the production of fruitbodies by *Thelephora terrestris* (Ehrh.) Fr. (Hacskaylo, 1965). Defoliation of birch trees instantly stopped the appearance of mycorrhizal sporophores which recommenced when a new 'crop' of leaves was produced (Last *et al.*, 1979). However, some mycorrhizal fungi have been observed to fruit in pure culture in the absence of their hosts (Giltrap, 1981) or in areas where their connections with a host have been severed (Modess, 1941). It is unclear whether these represent facultative biotrophs that, like the strain of *Boletus (Xerocomus) subtomentosus* L. ex Fr. reported by Lundeberg (1970) may be capable of an independent saprotrophic existence.

1.2.2 Mineral Nutrition

Although Frank (1894) first suggested that mycorrhizas play a role in the nutrition of the higher plant, the first experimental evidence to support this view was not published until 1937 when Hatch demonstrated that inoculated seedlings of *Pinus strobus* L. were larger and had greater amounts of nitrogen, phosphorus and potassium, both proportionally and totally, than uninoculated non-mycorrhizal seedlings. He suggested that these accumulations were attributable to the greater surface area for absorption of nutrients provided by a fungal sheath and attached hyphae and to other benefits, such as increased root longevity. Subsequently, roots with mycorrhizas have been shown to take up greater amounts of phosphate (Harley and McCready, 1950), potassium (Harley and Wilson, 1959) and zinc (Skinner, Bevege and Bowen, 1972) from nutrient solutions than non-mycorrhizal roots. In addition, various mycorrhizal fungi are able to utilise ammonium (Carrodus, 1966), nitrate (Lundeberg, 1970) and organic phosphates (Paterson and Bowen, 1968) and to produce acid phosphates *in vitro* (Ho and Zak, 1979).

Not all mycorrhizal fungi are equally effective at nutrient uptake. For example, Mejstřik and Krause (1973) found phosphate concentrations were enhanced in pines infected by *Suillus luteus* (L..ex Fr.) S.F. Gray, but depressed in pines colonised by *Cenococcum geophilum* Fr. Bowen (1973) considered that mycorrhizal fungi that produced mycelial strands would be more efficient than non-strand-formers at the uptake of slowly diffusible nutrients from soil as they can effectively explore a large soil volume and are effective at the translocation of solutes. Absorption and translocation to the host by mycorrhizal fungi of labelled phosphate from the soil has been demonstrated by Morrison (1962), Mejstřík and Krause (1973) and Skinner and Bowen (1974a).

1.2.3 Protection Against Soil-borne Pathogens

Levisohn (1954) noted that the mycorrhizal roots of pine and spruce appeared to resist infection by *Rhizoctonia* sp. whereas non-mycorrhizal roots were frequently damaged. She suggested that mycorrhizas deter infection by *Rhizoctonia* sp., recognising that soil conditions conducive to infection by the pathogen might inhibit the formation of mycorrhizas. After applying fungicides and nematocides to groves of pecan (*Carya illinoensis* (Wangenh.) K.Koch) suffering from feeder root necrosis, Powell, Hendrix and Marx (1968) observed that there appeared to be a correlation between increased tree vigour and the incidence of mycorrhizas formed by *Scleroderma bovista* Fr. Marx and Bryan (1969) later established that mycorrhizas of *S. bovista* deterred infection by *Pythium* species. Mycorrhizas have also been shown to be resistant to infection by both the zoospores and vegetative mycelium of *Phytophthora cinnamomi* Rands. (Marx and Davey, 1969a,b; Marx, 1970). Inoculation with *Laccaria laccata* (Scop. ex Fr.) Berk. & Br. was as effective as a fungicide drench in decreasing the numbers and severity of root lesions caused by *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. on the roots of Douglas-fir (Sinclair, Sylvia and

Larsen, 1982). Infection by *F. oxysporum* was also reduced by inoculation with *L. laccata* even before the formation of mycorrhizas (Stack and Sinclair, 1975). It seems that mycorrhizal fungi protect roots from some invading pathogens by producing antibiotics (Marx, 1969); by creating a passive physical barrier, the mantle and Hartig net, to infection (Marx and Davey, 1969a,b; Marx, 1970); and by possibly altering the nature of root exudates from, and the rhizosphere populations around, mycorrhizal roots (Marx, 1973).

1.2.4 Water Relationships

Worley and Hacskaylo (1959) found an inverse correlation between available soil moisture and the occurrence of the black mycorrhiza formed by *Cenococcum geophilum* which can grow at lower water potentials than some other mycorrhizal fungi (Mexal and Reid, 1973). Cenococcum-type mycorrhizas also survived water potentials as low as $^{-}55$ bars (Pigott, 1982a). The survival of an intact absorptive system through periods of drought could have obvious advantages to the host (Pigott, 1982b).

Mycorrhizal rhizomorphs of *Suillus bovinus* (Fr.)O.Kuntze have been demonstrated, by the use of tritiated water, to take up and translocate water to the host over significant distances, e.g. up to 8 cm (Duddridge, Malibari and Read, 1980). Roots of white oak (*Quercus alba* L.) seedlings incoulated with *Pisolithus tinctorius* (Pers)Coker & Couch elongated more rapidly during periods of water stress than their non-mycorrhizal counterparts and when re-watered the mycorrhizal plants recovered to higher water potentials than the non-mycorrhizal plants (Dixon *et al.*, 1980). It has also been suggested that the earlier development of bark, and the greater amount of air spaces in the cortex of the stems of *Betula pendula* Roth. when infected by *Amanita muscaria*

(L. ex Fr.)Pers. ex Hooker may contribute to plant establishment in both dry and waterlogged soils (Mason, Pelham and Last, 1977). Thus mycorrhizas may make a positive contribution to the host in many aspects of their water relationships.

1.2.5 Production of Growth Regulators

Levisohn (1953, 1956) observed that the growth of trees was stimulated by mycorrhizal fungi even before mycorrhizas were formed. In culture mycorrhizal fungi can produce auxins, cytokinins and giberellins (e.g. Ulrich, 1960; Crafts and Miller, 1974; Ng *et al.*, 1982; Pegg, 1973). The relevance of these observations to events in the field is largely unknown although the application of auxins to pine roots can induce morphological changes in roots similar to those caused by mycorrhizal infection (Slankis, 1973). Alexander (1981) suggested that the greater production of short roots and outgrowing buds by Sitka spruce (*Picea sitchensis* (Bong.)Carr.) when infected by *Lactarius rufus* (Scop.ex Fr.)Fr., compared to non-mycorrhizal plants, may be due to cytokinins or auxins produced by the fungus. The only actual demonstration of altered hormone levels in a plant due to mycorrhizal infection has been reported by Allen, Moore and Christensen (1980). In this case, the infection was endomycorrhizal, and by 1980 no similar report seemed to have been made for sheathing mycorrhizas.

1.3 Mycorrhizal Inoculation

The necessity of mycorrhizal infection for successful growth of trees was noted by Melin (1917) who observed that pine and spruce seedlings growing on recently drained peat bogs remained stunted and chloritic unless they were infected by mycorrhizal fungi. Similarly, attempts to establish nurseries and plantations of exotic pines in parts of the southern hemisphere, where there were no indigenous sources of mycorrhizal inoculum, frequently failed (Mikola, 1970, 1973). The same was true for the treeless grasslands, such as the prairies (Hatch, 1936; McComb, 1938) and sites where populations of mycorrhizal fungi had been depleted, e.g. by continuous agriculture or nursery practice (Trappe and Strand, 1969). Consequently, it was necessary to introduce mycorrhizal inoculum. Early inoculation procedures involved the importation of soil from established nurseries or plantations (Mikola, 1970) and although successful, the technique involved risking the importation of pathogens and weeds to the nursery. Other methods included the use of excised mycorrhizal roots or interplanted mycorrhizal seedlings (Sinclair, 1974; Trappe and Strand, 1969).

More selective techniques have involved the use of chopped up sporocarps (Mullette, 1976) or spores. Basidiospores have proved to be an effective inoculum in a number of studies (Theodorou and Bowen, 1973; Lamb and Richards 1974a,b; Marx and Bryan, 1975). Although the use of spore inocula is attractive, in that the spores are easy to handle and store (Lamb and Richards, 1974c; Marx, 1976, 1980), the range of fungi that can be used is limited to those that produce large numbers of easily collected sporophores. Little work has been done on the use of asexual spores though Lamb and Richards (1974a,b,c) found chlamydospores of three unidentified mycorrhizal fungi to be both less effective and to store less well than basidiospores.

Successful use of pure culture mycelial inoculum of *Pisolithus tinctorius* in fumigated nursery soil has been reported by Marx and

Bryan (1975) and found to be more effective than basidiospores of the same species (Marx, Bryan and Cordell, 1976). A wide range of fungi grown in pure culture have been used to inoculate container-grown conifers (Molina and Trappe, 1982a) and inoculum of *P. tinctorius* is now produced commercially for nursery inoculation in the south-eastern United States (Marx *et al.*, 1982). The use of pure culture inoculum is limited to fungi that can be grown successfully in the absence of their host. Comprehensive reviews on nursery inoculation with mycorrhizal fungi have been made recently by Marx (1981) and Molina and Trappe (1982a).

In selecting fungi for inoculation, Molina and Trappe (1982a) listed seven criteria to be considered. Two of these were the effectiveness as inoculum of a fungus and its effect on the growth and vigour of the host. These may vary considerably both within and between the species.

Marx (1981) reported that out of 13 fungi tested as basidiospore inoculum, only *P. tinctorius* and *Thelephora terrestris* were successful. Similar failure to establish infection by spores of several fungi has been reported by Trappe (1977). The use of pure culture inoculum has also had mixed success. For example, only *Laccaria laccata* and *Cenococcum geophilum*, out of a broad range of fungi and isolates, infected containerised western conifers (Molina, 1980; Shaw and Molina, 1980). Whilst *P. tinctorius* infected two species of pine well, under the same conditions *C. geophilum* failed completely and *Rhizopogon roseolus* (Carda) Hollos and *Suillus granulatus* (L. ex Fr.) O. Kuntz infected only a small proportion of root tips (Riffle and Tinus, 1982). The possible factors that may affect the ability of mycorrhizal fungi to infect the host will be discussed later.

When successful, mycorrhizal inoculation may result in variable effects on the host. Infection of *Pinus taeda* L. with *P. tinctorius* in fumigated nurseries has resulted in seedlings with up to twice the dry matter production of those infected by naturally occurring fungi, such as *T. terrestris* (Marx and Bryan, 1975; Marx, 1976). Increases in both the dry weight and numbers of plantable seedlings resulted from inoculation with *P. tinctorius*, *T. terrestris* and *C. geophilum* in fumigated soil, but in non-fumigated soil no weight increases resulted although there was some increase in the number of plantable seedlings (Marx, Morris and Mexal, 1978). *Laccaria laccata* has been demonstrated to selectively stimulate the top-growth of seedlings of Sitka spruce, Douglas-fir and ponderosa pine compared to other fungi or uninoculated controls (Mason *et al.*, 1983; Sinclair *et al.*, 1982; Trappe, 1977). Conversely, Trappe (1977) found that inoculation with *T. terrestris* inhibited the growth of Douglas-fir, western hemlock and ponderosa pine. Similar observations, where inoculation may result in plants with smaller tops than controls, have often been made (see Molina and Trappe, 1982a). Shaw, Molina and Walden (1982) suggested that the smaller size of spruce seedlings infected by *L. laccata* or *Hebeloma crustuliniforme* (Bull. ex St. Amans) Quel. compared to those either inoculated with *Amanita muscaria* or uninoculated controls, was due to the high demand on host photosynthate made by the first two fungi.

Relatively little work has been done on the effects of inoculation on the growth of seedlings in the soils or sites into which they would be transplanted. Prior inoculation of five pine species with *P. tinctorius* increased both their survival and growth after transplanting to re-afforestation sites (Marx, Bryan and Cordell, 1977). Similarly, inoculation with this fungus has also been shown to increase either growth or survival of trees planted into such sites as acidic coal spoils (Marx, 1979), prairie soils (Baer and Otta, 1981; Riffle and Tinus, 1982) and eroded sites (Berry and Marx, 1976). However, on a high altitude mine site tree growth was stimulated by inoculation with a *Suillus* sp. but not with *P. tinctorius* (Grosnickle and Reid, 1982). There was no improvement in the survival of Douglas-fir seedlings, after previous inoculation with *L. laccata* or *H. crustuliniforme*, when planted into dry, burned-over sites (Bledsoe, Tennyson and Lopushinsky, 1982). So, although there are clearly benefits, in improved tree growth, to be gained from mycorrhizal inoculation, a broad range of fungi and isolates will have to be tested to gain optimum benefit.

1.4 Factors Affecting the Formation of Mycorrhizas

1.4.1 Environmental Factors

Light intensities have been shown to have a marked effect on the development of mycorrhizas on tree seedlings (Wenger, 1955). Mycorrhizas of *C. geophilum* develop more plentifully than other mycorrhizal types in lower light intensities (Harley and Waid, 1955) and mycorrhiza formation has been observed to cease entirely at 6% of full daylight (Björkman, 1942). In that same experiment, illuminated plants, grown at high levels of nitrogen and phosphorus, had the same poor mycorrhizal development as heavily shaded plants. Björkman (1942) found that in each case the root systems had low levels of soluble carbohydrates. This led him to suggest that a surplus of such compounds is necessary for mycorrhiza formation. Considerable controversy was aroused by this hypothesis; it has been reviewed by Hacskaylo (1973), who pointed out that as mycorrhizal fungi rely on their hosts for carbohydrates, any factors that alter the availability of carbohydrates in the root may affect the formation of mycorrhizas. Consequently, a sufficient light intensity for a reasonable rate of photosynthesis must be deemed essential (Harley, 1969).

In other studies, high soil fertility has been found to depress mycorrhiza formation. For example, Shaw *et al*. (1982) found greater infection of Sitka spruce by *C. geophilum* in low than in high fertility treatments. Likewise, infection by *P. tinctorius* has been noted to be reduced by high soil fertility (Marx *et al*., 1982). In an earlier study, Marx, Hatch and Mendicino (1977) found that sucrose content of loblolly pine roots was positively correlated with mycorrhiza development, both were lower at high nutrient levels. By contrast, application of nitrogen as a foliar feed to shortleaf pine stimulated mycorrhiza formation by *P. tinctorius* (Dixon, Garnett and Cox, 1979) as did an application of $NaNO_3$ to inoculated *Quercus rubra* du Roi 40 days after planting (Beckjord, Adams and Smith, 1980). *Laccaria laccata*, however, has been shown to infect equally well at both high and low nutrient levels (Molina and Chamard, 1983).

Mycorrhizal fungi generally have optimum pH values of below 7.0 for growth (Harley, 1969). However, isolates of the same species can vary widely in their pH optima (Laiho, 1970). Richards (1961) observed that mycorrhiza formation was not always inhibited in neutral or slightly alkaline soils, provided the levels of nitrate were low. Where inhibition did occur, it could be corrected by adding chelated iron. Richards (1965) concluded that the effects of alkalinity on mycorrhizal establishment were not direct effects of pH *per se* but indirect effects on host nutrition by altering the availability of some nutrients. However, Theodorou and Bowen (1968) reported that *Rhizopogon luteolus* Fr. & Nordh. would not grow at pH 8.0 regardless of nitrate concentration and at pH 5.0 growth of the fungus was not depressed by nitrate but infection was depressed. This would seem to confirm the findings of Richards (1965), that the percentage of mycorrhizal infection was inversely proportional to the percentage of total nitrogen in the root system.

Temperature can influence the growth of, and the colonisation of roots by, mycorrhizal fungi (Hacskaylo, Palmer and Vozzo, 1965; Theodorou and Bowen, 1971). The latter authors found mycorrhizal formation to be optimal at 25°C, with a dramatic decline at 15°C, this temperature being closer to that of field soils in Australia at planting times. A small difference in temperature below 20°C could have a large effect on growth in the rhizosphere of some of the isolates tested. Marx, Bryan and Davey (1970) showed that the percentage of roots colonised by *T. terrestris* declined as temperatures increased above an optimum temperature range of 14 - 24°C, until at 34°C no roots were colonised. By contrast, *P. tinctorius* colonised increasingly more roots as the temperature increased. The high temperature tolerance of this fungus makes it an ideal candidate for inoculating seedlings to be planted onto coal wastes (Marx, 1981). Soil moisture may also influence the types of mycorrhizas formed on the host; this has been discussed in Section 1.2.4. When considering the effect of environmental factors on the formation of mycorrhizas, the factors should not be viewed in isolation as, for instance, pH may influence both the nutrients that are available and the form in which they exist.

1.4.2 Genotype/Genotype Interactions

Many mycorrhizal fungi exhibit broad host ranges but some are known, from field observations, to produce sporocarps with only a single host or genus (Trappe, 1962). However, Molina and Trappe (1982b), using pure culture synthesis tests, have found that some fungi that would be considered host specific from sporocarp associations can form well-developed mycorrhizas with some non-associated hosts. Fungi with broad host ranges showed, predictably, no incompatability in the formation of mycorrhizas with a variety of hosts. Little is known about host specificity in unsterile conditions, but as most of the fungi used in inoculation trials are likely to have broad host ranges, this is unlikely to be a problem. The provenance, or isolate, of the host or fungus may affect mycorrhizal development. Wright and Ching (1962) found a significant difference in the numbers of mycorrhizas formed on Douglas-fir seedlings of different provenances. Even within half-sib progenies of slash pine the host genotype influenced the formation of mycorrhizas by *P. tinctorius* (Marx and Bryan, 1971). The mycorrhizal establishment by *P. tinctorius* and *Suillus granulatus* on containerised *Pinus contorta* Engelm. and *Pinus ponderosa* Engelm. was also affected by the genotype of the host (Cline and Reid, 1982).

Although six isolates of *P. tinctorius* differed both culturally and in their effectiveness as inocula (Molina, 1979), four isolates of *L. laccata* did not differ significantly in the percentage of roots that they colonised on four conifer species (Molina, 1982). Using different provenances of birch, *B. pendula*, and isolates of *Amanita muscaria*, Mason (1975) found that both host and fungal genotype could affect the formation of mycorrhizas, e.g. by influencing the number of mycorrhizas that may be formed and the pattern of mycorrhizal branching.

1.5 Mycorrhizal Succession

So far, little attention has been paid to the possible role that interactions with either other mycorrhizal fungi or the soil microflora might have on both the formation of mycorrhizas and their replacement by other mycorrhizal types. Recent work at Edinburgh and elsewhere has indicated that there seems to be a succession of mycorrhizal fungi on trees as they age. In this section, the evidence for the observed succession will be reviewed.

Fruitbody succession

Sporophores of mycorrhizal fungi appearing in an experimental stand of birches (*Betula pendula* and *B. pubescens* Ehrh.), at the Institute of Terrestrial Ecology, Bush Estate, Penicuik, near Edinburgh, were observed to occur in a well ordered sequence in time and space (Mason *et al.*, 1982; Mason *et al.*, 1983a). In the first 2 years after planting, only fruitbodies of *Hebeloma crustuliniforme* and a *Laccaria* species were recorded. The following year (3), *Thelephora terrestris* was first noted, as were *Lactarius pubescens* (Fr. ex Krombh) Fr. and *Inocybe lanuginella* (Schroet) Konrad & Maubh in the fourth year. From the fifth year onwards, further species of *Hebeloma* were recorded, species of *Leccinum* and *Cortinarius* first appeared in the sixth year and in the tenth year three species of *Russula* were first recorded. Although *Amanita muscaria* is frequently associated with birch (Trappe, 1962) no fruitbodies of this fungus have yet appeared in this stand at Edinburgh, which is now 12 years old.

Many of the fruitbodies were concentrated in 'fairy rings' around the trees. Typically, fruitbodies first appeared as a ring close to the trunk and in subsequent years the ring moved progressively outwards

(Ford, Mason and Pelham, 1980). Thus, for example, in the sixth year after planting, rings of *H. crustuliniforme* had a mean radius of 73 cm from the trunk, *L. pubescens* a radius of 69 cm, with *Leccinum* sp. only 27 cm from the tree (Mason *et al.*, 1983a). Not all of the fruitbodies appeared in rings, however; a *Laccaria* sp. tended to be arranged in clumps possibly following the lines of secondarily thickened roots (Ford *et al.*, 1980).

Rings of mycorrhizal sporophores have also been observed around trees by Horn (1933), Becker (1956) and Tominaga (1975). The last two authors noted the outward movement of the rings with time and Becker described concentrically arranged rings of several fungi around Scots pine (*Pinus sylvestris* L.)

Likewise, similar sequences of mycorrhizal fruitbody production in time have been reported for other trees apart from birch. Trappe and Strand (1969) observed abundant fruiting of *H. crustuliniforme*, *Laccaria laccata*, *T. terrestris* and *Inocybe lacera* (Fr.) Quel. in nursery beds of Douglas-fir, *I. lacera* being frequently present in rings. Marked associations of different fungal fruitbodies with differently aged stands of *Pinus radiata* D. Don have been recorded by Chu-Chou (1979) in New Zealand. *H. crustuliniforme* was abundant in nurseries but not after outplanting. *Rhizopogon luteolus*, *R. roseolus* and *L. laccata* were frequently found in nurseries and 3-5 year old stands, but rarely in stands greater than 10 years of age; *Suillus* sp. and *Inocybe* sp. were most often seen after the trees were 5 years old; *A. muscaria* and *Scleroderma verrucosum* Vaill ex Pers. were never found in stands younger than 10 years old and were most abundant when the trees were older than 15 years. Similarly, fruitbodies of *H. crustuliniforme* were only associated with Douglas-fir in nurseries and sporophores of *A. muscaria* only observed in plantations of the same tree that were over 12 years old (Chu-Chou and Grace, 1981). The numbers of sporophores of *A. muscaria* produced in stands of *Pinus patula* Schl. & Cham in India increased dramatically with the age of the stand from 5 to 16 years (Last *et al.*, 1981).

There is therefore considerable evidence for a succession of fruitbodies of mycorrhizal fungi around trees as they age, some fungi being more typically associated with younger trees and others with older trees. Harper and Webster (1964), in an analysis of a coprophilous fungal succession, demonstrated that fungal successions should not be interpreted on the basis of fruitbodies alone. Fungi require different minimum periods of vegetative growth before they can produce a fruitbody. Therefore, Hudson (1968) considered that in analysing successions two questions should be asked, (a) are all the fungi present on the substrate initially? (b) do the fungi arrive and colonise at different times or is the sequence observed simply due to the time needed to produce a fruiting structure?

Warcup (cited in Mason *et al.*, 1983a) found that most mycorrhizal fungi could be isolated from the mycorrhizas beneath their fruitbodies. Tominaga (1975) observed that mycorrhizas of *Tricholoma matsutake* (S. Itoe & Iman) Sing. were most common either directly beneath, or just beyond, the rings of their fruitbodies. The association of mycorrhizas with their fruitbodies is to be expected and does not provide evidence of a succession of the mycorrhizas themselves. Isolations from the mycorrhizas of *Pseudotsuga menziesii* (Mirb.) Franco and *Pinus radiata* showed that, in both instances, *H. crustuliniforme* could only be isolated from nursery seedlings and *A. muscaria* only from trees older than 13 years (Chu-Chou, 1979; Chu-Chou and Grace, 1981).

This confirmed the author's observations on the production of fruitbodies, and established that not all the fungi were present on the 'substrate', i.e. the host, at an early stage. Deacon, Donaldson and Last (1983) working on the experimental birch stand at Edinburgh mentioned earlier, have established that there is a spatial sequence of mycorrhizas around the trees and that there are behavioural differences between the fungi associated with young trees and with older trees. Dissection of soil cores collected at different distances from the trunk of an 8-year-old tree revealed differences in the distribution of mycorrhizal types. Leccinum-type mycorrhizas were confined within a 50 cm radius from the tree in the year of sampling when the trees were 8 years old, Hebeloma-type mycorrhizas were found predominantly beyond this distance. Mycorrhizas of *L. pubescens* were fairly evenly distributed, though the greatest numbers were found at 50 cm distance. The distribution of mycorrhizas thus broadly reflected the patterns of fruitbody appearance around the trees described earlier. However, when Deacon *et al.* (1983) grew birch seedlings in soil cores collected at the same positions around the tree, only *Hebeloma* and four other unknown mycorrhizal types infected the seedlings. There was no infection by the *Leccinum* sp. or *L. pubescens*. Seedlings were then grown in soil cores taken from directly beneath fruitbodies of fungi from different 'stages' of the succession, i.e. *Laccaria* sp., *Hebeloma* sp., *Inocybe lanuginella*, *L. pubescens* and *Leccinum* sp. Although the mycorrhizas of each fungus were present in all, or most, of the cores, only *Laccaria*, *Hebeloma* and *Inocybe* readily formed mycorrhizas on the seedlings grown in the cores. Similar results were obtained when birch seedlings were grown in soil supplemented with pure culture vermiculite-peat inocula of a similar range of fungi, including *A. muscaria* which, like the *Leccinum* sp., did not infect the seedlings.

Deacon *et al.* (1983) thus distinguished between 'early-stage' mycorrhizal fungi that could infect seedlings from inoculum, either added or present in the soil, and 'late-stage' fungi that could not do so. This distinction was only possible in soil; in bixenic culture all of these fungi readily formed mycorrhizas with birch seedlings. Further evidence for this broad distinction is presented by Fox (1983). Birch seedlings grown in soils supplemented by basidiospores of two species each of *Inocybe* and *Hebeloma* and with *L. pubescens* or *Leccinum roseofracta* Watling formed mycorrhizas only with the *Inocybe* and *Hebeloma* species. Both the *Lactarius* and *Leccinum* species failed to infect the seedlings.

Reported failures in establishing infection by some fungi in unsterile conditions could be partly due to the choice of fungi used, i.e. whether they were 'early-stage' or 'late-stage' types (*sensu* Deacon *et al.*, 1983). For instance, Marx (1981) attempted, without success, to synthesise mycorrhizas on two pine species using, as inoculum, the basidiospores of three species of *Amanita* and three of *Lactarius*. Inoculation of pine seedlings with *A. muscaria* and *Suillus* (*Boletus*) *granulatus* by Rosendahl (1942) did not result in any mycorrhiza formation, although inoculation with *Boletus felleus* Fr. did so. Similarly, *Amanita pantherina* (DC. ex Fr.) Secr. and *Astraeus pteridus* (Shear) Feller failed to infect containerised Sitka spruce but *Laccaria laccata* and *Cenococcum geophilum* did so successfully (Shaw and Molina, 1980). However, Shaw *et al.* (1982) found that *A. muscaria* would infect Sitka spruce seedlings when they were grown in a vermiculite-peat mixture, albeit unsterile. Whilst these reports could be taken as indirect evidence to support the concept of 'early' and 'late-stage' fungi, in some instances inoculation attempts fail with fungi that are known under similar conditions to be able to infect seedlings; for example, *P. tinctorius* (Molina, 1980; Shaw and Molina, 1980; Shaw *et al.*, 1982) and *Laccaria laccata* (Marx, 1980). Consequently, other factors that affect the formation of mycorrhizas should not be overlooked. Nevertheless it now seems essential that in selecting fungi for mycorrhizal inoculation, a further criterion, as suggested by Mason *et al.* (1983a) should be considered, namely that fungi from early in the succession should be chosen - those capable of infecting young seedlings.

1.6 The Persistence of Mycorrhizal Inoculants

If selected isolates of mycorrhizal fungi are to be used in artifical inoculation programmes, it is desirable that the inoculant fungus persists on, and spreads with, the root system for as long as possible after transplanting (Lamb, 1979). But there has been little work on the fate of artificially established mycorrhizas after transplanting or of the factors involved in the replacement of one type by another.

Benecke and Göbl (1974) found that mycorrhizas present on seedlings of *Pinus mugo* Turra. from a nursery were totally superseded by indigenous mycorrhizal types when transplanted to a sub-alpine soil. The entire replacement of a limited nursery mycoflora by a diverse array of unidentified mycorrhizal fungi on the roots of *Pinus* species after transplanting has also been demonstrated by Lamb (1979). Likewise, Bledsoe *et al.* (1982) found that Douglas-fir seedlings, although inoculated with *H. crustuliniforme* or *L. laccata* before transfer to the field, had all their new roots colonised by indigenous mycorrhizal fungi within 5 months. The inoculant type mycorrhizas were confined to the old root system.

Further examples of the fate of inoculated mycorrhizas are provided by Marx *et al*. (1977) who reported that whilst *P. tinctorius*, inoculated onto seedling pine, still persisted 2 years after planting in re-afforestation sites, increasing proportions of the root system were colonised by naturally occurring symbionts. Similarly, three or four morphological types of mycorrhiza were found on the new roots of inoculated pines planted into a prairie soil (Riffle and Tinus, 1982). After 4 years' growth on a high altitude mine site, trees initially inoculated with *P. tinctorius* or *C. geophilum* no longer bore mycorrhizas of these types on their root systems (Grossnickle and Reid, 1982). Trees inoculated with *Suillus granulatus*, though, still had the inoculant types present.

Marks and Foster (1967) observed sequences of two and three mycorrhizal types on individual roots of *Pinus radiata*; they concluded that in order for one type to replace another an interruption to the growth of the root must occur. This could happen when nursery grown seedlings are planted into sites where environmental conditions are very different from the nursery. Marx *et al*. (1977) considered that a fungus, or fungi, most adapted ecologically to a particular site will come to dominate the root system.

Mason *et al*. (1983a) found that birch seedlings inoculated in bixenic conditions with *Paxillus involutus* (Batsch) Fr. or *Hebeloma sacchariolens* Quélet had only these fungi present on their root systems after one growing season in four field soils. At the end of the second growing season, however, *P. involutus* accounted for, at most, only 50% of the mycorrhizas present on the root systems of its treatment group. Inoculated *H. sacchariolens* was still the dominant type in three of the soils but in the fourth it had been completely replaced.

Soil type, therefore, seems to have some influence on the persistence of mycorrhizal inoculants.

In the same investigation (Mason *et al*., 1983a), seedlings were also propagated bixenically with *A. muscaria*, but no new mycorrhizas were formed by this fungus after transplanting into the unsterile soils. The new roots were all colonised by the fungi that infected the control seedlings. The authors suggested that this represented a further distinction between 'early-stage' and 'late-stage' fungi (*sensu* Deacon *et al*., 1983). The 'late-stage' types being unable to form mycorrhizas with seedlings in unsterile soil were therefore unable to spread down a growing root. 'Early-stage' fungi, on the other hand, readily spread along a growing root system to such an extent that, for the first year at least, indigenous mycorrhizal types were completely excluded from infecting new roots. This last point would clearly be a desirable characteristic for a selected fungus.

Unlike saprophytic fungi, which are thought normally to rely on successive lateral colonisation of roots from the soil (Taylor and Parkinson, 1961), mycorrhizal fungi, once established, may grow along a root externally or, in the case of pine and spruce, internally (Robertson, 1954; Laiho and Mikola, 1964; Theodorou, 1980). After a period of dormancy, root growth may begin in advance of growth of the fungus (Wilcox, 1968) and new laterals, for instance, may break away from the fungal mantle (Robertson, 1954). The growth rate of hyphae of *Rhizopogon luteolus* has been measured at 1.5 mm/day when the roots of its host, *Pinus radiata*, were growing at 3-4 mm/day (Bowen and Theodorou, 1973). Consequently, a vigorously growing root would easily outgrow fungal hyphae, a point also noted by Wilcox (1968). These roots may then become colonised by other mycorrhizal fungi from propagules in the soil (Theodorou, 1980), so the sequences of mycorrhizal types observed by Marks and Foster (1967) on individual roots could occur. Greenhalgh (1976) considered that the different growth rates of mycorrhizal fungi along and around roots may play a major part in the replacement of one type by another.

Mycelial strands are produced by many mycorrhizal fungi and may penetrate the soil or grow ectotrophically along roots (Skinner and Bowen, 1974b; Ashton, 1976). Garret (1951) suggested that the major role of mycelial strands in root-inhabiting fungi is to initiate infection. Strands have been observed to spread from centres of mycorrhizal infection to colonise roots some distance away (Young, 1937; Robertson, 1954) and similarly to be associated with the spread of infection in nurseries (Levisohn, 1956). The suitability of mycelial strands for their role in spatial dispersal and as agents of secondary infection has recently been emphasised by Chilvers and Gust (1982). Mycelial strands may grow at 2-3 times the rate of individual hyphae, those of *Rhizopogon roseolus* can grow at 2-4 mm/day along roots of *P. radiata* (Skinner and Bowen, 1972). This potential ability to keep up with root growth may give strand-forming mycorrhizal fungi a competitive advantage over non-strand forming types in persisting on root systems.

How interactions with other soil microbes affect the persistence of a mycorrhizal fungus or its ability to infect is little known. Bowen and Theodorou (1973) found that *Pseudomonas* sp. inhibited the growth of *R. luteolus* both in the rhizosphere and around inert fibres and they suggested that this was due to antibiosis. Conversely, Theodorou (1976) reported that the length of *P. radiata* roots colonised by *Corticium bicolor* Peck (= *Piloderma croceum* Erikss & Hjortst) was increased in the presence of a *Bacillus* sp. The change in mycorrhizal types with time reported by Lamb (1979) was thought by Bowen and Rovira (1976) to be probably due to changes in the overall microbial balance. Likewise, they proposed that the domination of coal spoils by *P. tinctorius* (Schramm, 1966) and its absence from more fertile sites may be due to its sensitivity to organisms present in the latter sites that are absent from coal spoil.

Finally, both Last *et al.* (1983) and Mason *et al.* (1983a) suggest that in selecting a fungus to persist after inoculation, and also when considering mycorrhizal succession, the interactions between host, fungus and soil microbes should be taken into account, each of these three factors being subject to the influence of the environment.

1.7 Aims and Objectives

The aims of the present study were to further the work that has been done on the mycorrhizal succession observed on birch (*B. pendula*) trees at Edinburgh. Whilst contributing further to an understanding of the ecology of mycorrhizal fungi, the work was expected to have practical implications in inoculation and afforestation programmes. The work has concentrated on two major areas:

1. Monitoring the fate of artificially established mycorrhizas of birch after transplanting into field soils and, in particular, the role of competition from other mycorrhizal fungi on the persistence of inoculant types.
2. Examination of the factors that may affect the establishment of 'late-stage' fungi on birch seedlings in unsterile conditions, with particular reference to the role of mycelial strands.

SECTION II

Materials and Methods

2.1 Fungal Inoculum

2.1.1 *Fungal Isolates*

Isolates of mycorrhizal fungi were obtained either from fruit-bodies or directly from mycorrhizas (Section 2.1.2). They were maintained as actively growing colonies on Hagem's agar (Mason, 1980) or half-strength potato dextrose agar (PDA/2) incubated in darkness at 20°C. The sources of isolates used experimentally are listed in Table 2.1.1; all were obtained from sporophore tissue.

2.1.2 *Isolation from Mycorrhizal Roots*

Mycorrhizas were sorted into distinct types (Section 2.5.2b) and cut into 5 mm lengths. These were then shaken in 28 cm^3 sterile distilled water for 5 min before being immersed in hydrogen peroxide (30% w/v H_2O_2) for 10-20 sec. (Zak, 1969a). The roots were given two further rinses in sterile distilled water and then transferred to plates of PDA/2 or Hagem's agar.

2.1.3 *Vermiculite-peat Inoculum*

Bulk cultures of isolates were grown in wide-necked 500 cm^3 Erlenmeyer flasks containing 250 cm^3 of a vermiculite-peat mixture (9 : 1, v/v) supplemented with 180 cm^3 of modified Melin-Norkrans solution (Mason, 1980). After autoclaving for 30 min at 121°C, flasks were inoculated with three 4 mm^3 agar blocks cut from the edge of a growing colony of the required isolate. The flasks were then incubated at 20°C; during incubation the flasks were shaken occasionally to ensure thorough colonisation of the medium by the fungus. Generally, the flasks were completely permeated by the fungus after 12 to 16 weeks.

TABLE 2.1.1: Origin and source of fungal isolates used experimentally; all isolates except * were obtained from birch.

Fungus	Isolate code (I.T.E.)	Date of isolation	Locality	O.S. reference	Origin**
Amanita muscaria	71	September 1974	Loch of the Lowes	NO 046447	b
" "	183	October 1981	Coal spoil, Newtongrange	NT 330649	b
Hebeloma sacchariolens	4	October 1979	Bush Estate (tree K8)	NT 246638	b
Laccaria proxima	4	September 1979	Coal spoil, Roslin	NT 263635	b
Lactarius pubescens	6	July 1978	Bush Estate (tree N2)	NT 246638	b
" "	8	September 1982	Bush Estate (tree K2)	NT 246638	a
Leccinum scabrum	6	October 1980	Pentlandfield	NT 253646	a
Paxillus involutus	16	August 1979	Coal spoil, Newtongrange	NT 330649	b
" "	32*	October 1981	Gladhouse	NT 287536	b
Thelephora terrestris	1	- -	-	-	c

* Probably from *Picea sitchensis*
** Origin of isolates = a, the author; b, Dr. P.A. Mason; c, Dr. R.M. Jackson

2.2 Plant Material

2.2.1 Collection of Birch Seed

Seed was collected in October 1980 from an 11-year-old tree of *Betula pendula* (designated 12·5A2) growing on the Bush Estate, Penicuik, Midlothian. The seed was dried at room temperature and then stored in sealed paper envelopes in the dark at 2 ± 1°C. Most experiments were done using this seedlot. However, a second collection, in September 1982, of seed from the same tree was necessary.

2.2.2 Separation of Seed

Seed material was rubbed between the fingers to break up the catkins and remove the seed wings; then full and empty seeds were separated by a modification of the method of Baldwin (1932). Seed was added to about 200 cm^3 of methanol and those that floated were discarded; all the discarded seeds examined were found by dissection to be empty. The methanol was carefully decanted off the seeds that had sunk and these were then dried thoroughly on filter paper for 1-2 hours. Seed that had been separated in this way was used immediately and not returned to storage (Barnett, 1971).

2.2.3 Germination of Birch Seed

Aseptically germinated birch seedlings were produced by the method of Pelham and Mason (1978). Seeds were surface sterilised by shaking in 7 cm^3 of hydrogen peroxide solution (30% w/v in water) for 30 min. The seed was then aseptically transferred to plates of water agar (10 g agar l^{-1}). The seeds were then incubated in aluminium foil-lined cabinets (1200 x 600 x 450 mm) with continuous illumination from three 'Gro-lux' fluorescent strip lights (40 W). Temperatures within the cabinets were 26 ± 2.0°C and light intensity at plant level was 10 Wm^{-2}, measured with Kipp's solarimeter. Most viable seed germinated within 14 days. Separation of seed in methanol raised the mean percentage germination of the seedlot from approximately 1% to 70%; after two years storage, however, the mean percentage germination of the seedlot had declined to 20% after separation. Samples of discarded seed from separation did not germinate after being surface sterilised and incubated in the same manner as above.

2.3 Synthesis of Mycorrhizas

2.3.1 Pure Culture Synthesis

Fungal isolates were tested for their ability to form mycorrhizas with birch in bixenic culture (Mason, 1980). An aseptically germinated birch seedling was added to a flask of vermiculite-peat medium which had been inoculated 14 days earlier with the selected isolate (Section 2.1.3); it was thus possible to see if the fungus was growing actively before adding a seedling. The flask was then incubated in a growth cabinet (Section 2.2.3) for 10-12 weeks, when the seedling was removed and assessed for the formation of mycorrhizas (Section 2.5.2b).

2.3.2 Production of Large Numbers of Mycorrhizal Seedlings for Transplanting

The method of Mason *et al*. (1983b) was followed in order to produce mycorrhizal seedlings, inoculated in bixenic culture, for subsequent transplanting.

Horticultural plastic seed trays (222 x 165 x 57 mm), without drainage holes, were packed with 140 to 150 grey polystyrene tubes (Figure 2.3); each 75 mm long, 13 mm internal diameter and split along one side (Telcon Plastics Ltd). For each tray containing tubes, a separate empty seed tray and a fitting transparent propagator lid

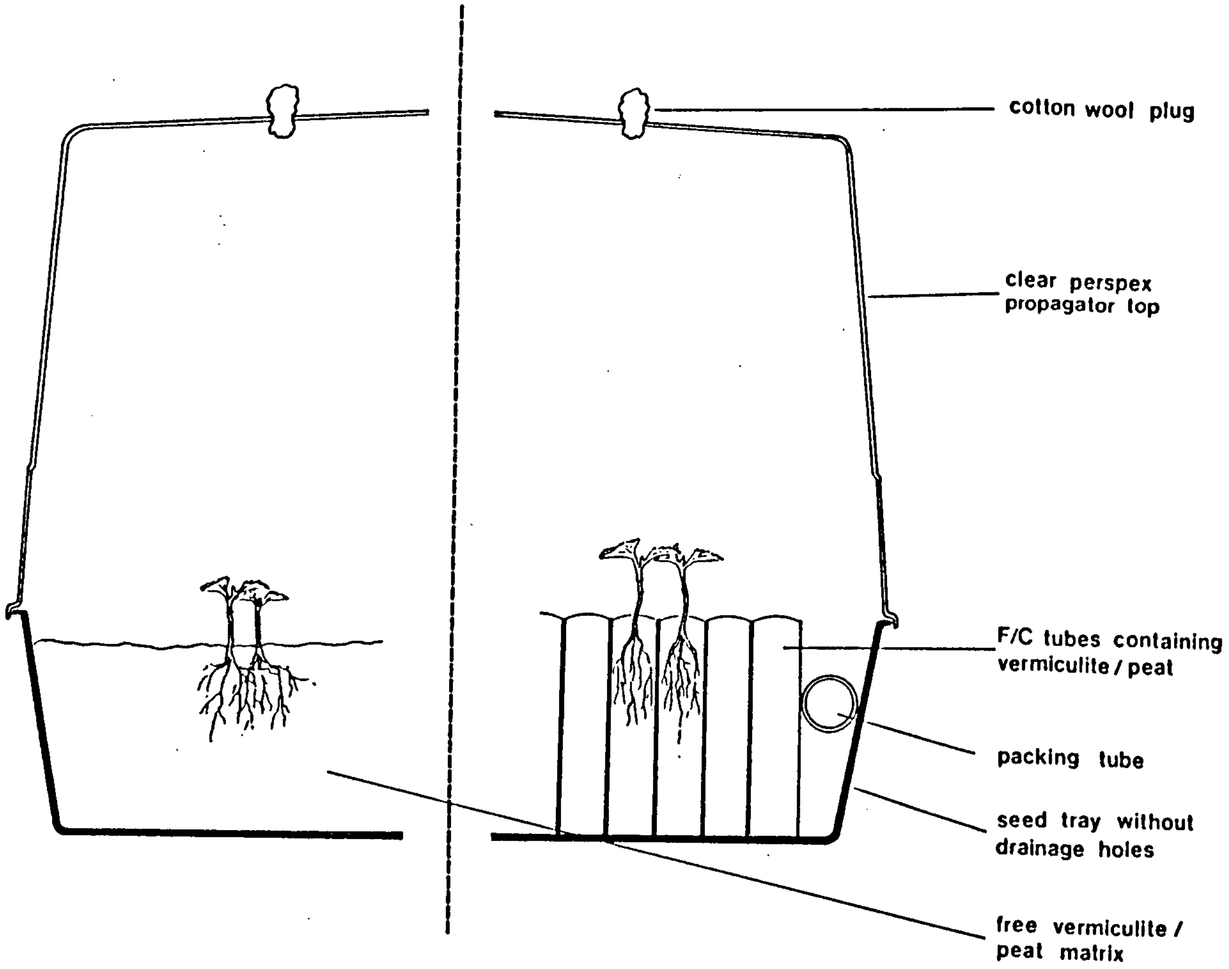

FIGURE 2.3: Closed system for establishing sheathing mycorrhizas during propagation (reproduced by kind permission of Dr. P.A. Mason).

(Stewart Plastics Ltd), air vents plugged with cotton wool, were required. Each of these was separately wrapped, sealed in polyethylene bags and sterilised by gamma irradiation (2.5 Mrad).

Further manipulations, requiring two people, were made in a laminar flow cabinet supplying filtered air. The empty seed tray was used to mix 250 cm^3 of vermiculite-peat inoculum with 750 cm^3 of autoclaved vermiculite-peat supplemented with 450 cm^3 of modified Melin-Norkrans solution. The tubes in the remaining tray were then filled with the mixture using a long-handled spoon. An aseptically germinated birch seedling was transplanted into each tube and the propagator lid was fitted and sealed with autoclave tape (Figure 2.3.). Non-mycorrhizal seedlings were produced by the same technique except that no inoculum was required and no glucose was added to the modified Melin-Norkrans solution supplementing the correspondingly larger volume of uninoculated vermiculite-peat medium.

The propagators were incubated for 6-8 weeks in growth cabinets with continuous lighting (Section 2.2.3). The seedlings were then 'hardened-off' by removing the propagator lids for increasingly longer periods each day before the propagators were transferred to a heated glasshouse; if the seedlings were to be transplanted to the field they were then transferred to an unheated glasshouse for a short period. The seedlings were assessed for mycorrhizas and shoot growth by taking a random sample of usually 5-10 seedlings from each propagator. At transplanting the plastic sleeve, in which each seedling was grown, was removed with minimal disturbance to the root 'plug'.

2.4 Plant Cultivation

2.4.1 Growth Conditions

The seedlings that were transplanted to pots of soil for further cultivation, referred to as pot culture, were propagated in either a heated glasshouse or a growth room. In all experiments, plastic plant pots were used; the size of pots used in individual experiments are given separately.

(a) Heated glasshouse:

Natural daylight in the glasshouse was supplemented by seven 400 W mercury vapour lamps to give a daily photoperiod of 20 h. Pots containing seedlings were placed on a wire mesh bench, 74 cm from ground level, and light intensities at plant level from the lamps alone ranged from 10 Wm^{-2} to 3 Wm^{-2}, depending on the position on the glasshouse bench. Temperature in the glasshouse was maintained at about 20°C, but the temperature ranges for individual experiments are given separately because temperature varied throughout the year. The glasshouse was fitted with automatically controlled vents and an extractor fan and two 16 kW fan heaters, with polythene ducts, in order to maintain temperatures. Plants within the glasshouse were watered daily.

(b) Growth room:

Lighting in the insulated growth room (4.1 x 2.1 x 2.1 m) was supplied by four 400 W mercury vapour lamps giving a daily 16-hour photoperiod. Pots were placed on a wire mesh bench 9 cm above ground level; light intensities at plant level ranged from 16 Wm^{-2} to 31 Wm^{-2}. Temperature within the growth room was maintained at 16 ± 3°C. Plants in these conditions were watered when necessary.

2.4.2 *Soil*

Fresh soil for pot culture was collected as required. If necessary it was dried at room temperature until friable enough to pass through a 1 cm-square mesh; the soil was never allowed to dry completely. The soil used predominantly for pot culture experiments was a freely drained brown earth of pH 6.4 derived from fluvio-glacial sands and gravel originating from Carboniferous sediments. It was collected from a site on the Bush Estate (O.S. reference NT 246638) which was at least 10 m from any trees. The soil had previously borne potatoes and soft fruit; it is referred to as 'non-mycorrhizal' Bush soil because it did not contain any tree roots. Where other soils were used in experiments, they are described in the appropriate sections.

When necessary, the soils were supplemented with vermiculite-peat inoculum, in usually a 3 : 1 ratio (v/v) of soil to inoculum; both were thoroughly mixed together in a clean enamel tray.

2.5 Design, Sampling and Analysis of Experiments

2.5.1 *Experimental Design*

In pot culture experiments where factorial combinations of treatments were being tested complete randomised blocks were prepared; otherwise experiments were completely randomised without blocks. The layout of such experiments in the glasshouse and growth room was determined by the available bench size, but pots were always spaced at least 3 cm apart to reduce the possibility of inoculum being transferred from one to another.

2.5.2 Assessment of Experiments

(a) Sampling methods:

Pot-grown seedlings 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 that were recorded included the following: height; basal stem diameter; dry weight, determined by drying to constant weight at 80°C; leaf area, estimated by tracing around leaves and counting graph paper squares within the outline.

The pots, with their root systems intact, were then stored in the dark at 2 ± 1°C until they could be assessed, this generally being within 10 days. For seedlings sampled from field experiments, the root systems were assessed immediately. The roots were washed free of soil under a gently running flow of tap water. However, cores of soil collected for direct root examination were washed under a tap on a 500 μm mesh sieve. If necessary, the core was gently broken by rubbing it against the mesh. The residue was then washed off the sieve into a petri-dish of water for examination.

(b) Characterisation of mycorrhizas:

Mycorrhizal types were distinguished on the basis of colour, gross morphology, texture of the sheath surface and the macroscopic and microscopic characteristics of external mycelium and mycelial strands, when present (Zak, 1969b). For a more detailed examination of mycorrhizas by scanning electron microscopy, specimens were fixed in a 2% (v/v) glutaraldehyde solution and then dehydrated in an acetone series (25% - 50% - 75% - 100% x 2). The specimens were then critical-point dried with liquid carbon dioxide as the transitional fluid; they were then mounted on aluminium stubs, sputter-coated with 275 Å gold (Au) in a Nanotech SEMPREP II and examined in a Cambridge

Stereoscan MkIIA scanning electron microscope, at 30 KV, γ1 and 150 μm final aperture. All the mycorrhizal types referred to in this thesis are described in Appendix 1, and a reference sample of each type is preserved in 2% glutareldehyde solution at 2 ± 1°C at the Institute of Terrestrial Ecology, Bush Estate, Penicuik, Midlothian. Where possible, mycorrhizal types were identified by comparison with those formed in bixenic culture with cultures of known origin and by constant association with fruitbodies in pot culture or in the field. Mycorrhizas that were dark brown or black and locally collapsed with a wrinkled or fissured surface were assumed to be dead (Harvey, Larsen and Jurgensen, 1976). Uninfected root tips were distinguished by the absence of a fungal sheath or Hartig net.

(c) Quantitative assessment of mycorrhizal infection:

Numbers of root tips of different mycorrhizal types, dead mycorrhizas and uninfected roots were counted under a stereoscan microscope (x 6 - x 50 magnification); 'scars' on the sides of mycorrhizas were not counted. Frequent checks on the different mycorrhizal types were made by transmission light microscopy with reference to the preserved types mentioned above.

The distribution of mycorrhizas along a root system was assessed by laying entire root systems on a sheet of glass such that the roots were at right angles to an underlying linear scale. The root system was then cut into pieces, generally 3 cm long, from the base of the stem to the distal tip of the root system; each piece was then counted separately.

Large root systems or portions of them were subsampled by a modification of the technique described by Marks, Ditchburne and Foster (1968). A 10 cm square plastic tray was divided into 100, 1 cm squares by scoring the base with a razor; each square was individually numbered. The sample to be counted was cut into small fragments ($\leqslant$ 5 mm length) with a scalpel and distributed evenly in the tray in about 20 cm^3 of water. Root tips within ten of the squares, selected randomly, were then counted as described earlier and the total was multiplied by ten to give an estimate for the entire sample. If less than 200 root tips were counted in the first ten squares then a further ten were counted; if this total had not been reached after counting thirty squares then the entire sample was counted.

2.5.3 Statistical Analysis

Generally, results were subjected to analysis of variance, and in order to ensure that they met the requirements of this (Sokal and Rohlf, 1969) they were transformed to log (x+1) or, in the case of percentages, to angles. Providing that the variance ratio test was significant, differences between the means were found by calculating the least significant difference (LSD, p=0.05) or, where there were uneven numbers of replicates between treatments, by using the Student-Newman-Keuls multiple range test (Sokal and Rohlf, 1969). Transformed data, on which the significance of any difference was calculated, are presented together with the arithmetic means; de-transformed means are not presented.

SECTION III

Experiments on the Distribution, Survival and Effectiveness as Inocula of the Mycorrhizal Roots of Birch

3.1 Introduction

In this section are described preliminary investigations into procedures for determining root distributions around birch trees growing in an experimental field plot. Also described are experiments on the abilities of existing mycorrhizas to act as inocula for mycorrhizal establishment on seedling roots, and on the survival of mycorrhizal inoculum in soil in the absence of a suitable host. These experiments are appropriately grouped together because they address several questions relevant to the studies presented later in this thesis and in published work.

3.2 Distributions of Mycorrhizas in a Young Experimental Birch Plot

An experimental plot of birches had been set up in 1975 on a previously tree-less site termed 'Farfield' (OS reference NT 263633) south of Edinburgh (P.A. Mason, personal communication); the soil was a brown earth. The experiment, laid out in a randomised block design, comprised four birch clones (two of *Betula pendula* and two of *B. pubescens*) which had been grown for a season in a glasshouse in pots of soil from two sites - Farfield and Bush Estate; the two clones of each tree species were derived originally from seedlings of the same seed source. The occurrence of fungal fruitbodies around the trees had been recorded each year up to, and including, 1979 (P.A. Mason, J.P. Pelham and F.T. Last, unpublished), and notable and consistent differences in this respect were found between the different treatments (Table 3.2.1).

By 1980, no records had been made of the numbers and types of mycorrhizas on the root systems. The experimental plot therefore provided an opportunity to investigate mycorrhizal distributions on the

TABLE 3.2.1: Mean numbers of fruitbodies of three mycorrhizal fungi produced in 1978 around two clones of *B. pubescens* (9.3D and 9.3G) propagated in two soils; data transformed to log(x+1) for analysis (in parentheses)*.

	Laccaria tortilis		*Laccaria proxima*		*Inocybe petiginosa*	
Soil	Clone 9.3D	Clone 9.3G	Clone 9.3D	Clone 9.3G	Clone 9.3D	Clone 9.3G
Bush	0.0	39.0	0.4	0.9	7.0	2.0
	(0.000)	*(1.334)*	*(0.135)*	*(0.285)*	*(0.886)*	*(0.416)*
Farfield	0.5	0.3	0.4	6.0	11.0	3.0
	(0.185)	*(0.119)*	*(0.135)*	*(0.841)*	*(1.069)*	*(0.648)*
L.S.D. (p=0.05)	*(0.532)*		*(0.365)*		*(0.472)*	

* Source of table: P.A. Mason, J. Pelham and F.T. Last, unpublished.

root systems and to attempt to relate the distributions to the known occurrence of fungal fruitbodies. This preliminary experiment thus had three objectives, as follows:

1. To find a satisfactory method of determining root distributions around trees in the field.
2. To investigate whether the differences in fruitbody production between the trees accurately reflected differences in mycorrhizal status.
3. To determine whether the spatial distributions of fruitbodies were reflected in the spatial distributions of different mycorrhizal types.

Four trees were selected within one block of the field experiment; they were designated W65, W62, Q68 and N62 and represented each clone of *B. pubescens* propagated in each soil type, as follows.

Tree No.	Clone	Soil used during propagation
W65	9.3D	Farfield
W62	9.3G	Farfield
Q68	9.3D	Bush
N62	9.3G	Bush

On 14 November 1980, two soil cores, 8 cm deep and 2 cm diameter, were taken at each of five distances (20, 35, 50, 65 and 80 cm) along four radii marked out from the base of each tree; a single core was also taken at 10 cm distance. The radii were separated by one angle of 120° and three of 80°. Twenty-five other cores, to act as controls, were collected at random within the block but at least 1 m distance away from any tree. Five of the control cores and a core from every distance

on each of the radii were dissected and examined for mycorrhizas (Section 2.5.2); the remaining cores were used for a seedling bioassay. For bioassay, the cores were inverted, wrapped in a clean strip of polyethylene, 8 x 15 cm, randomised and sunk into a tray of moist sand; a pre-germinated birch seedling was then planted on top of each core. Inversion of the cores was done to reduce competition from weeds and also to avoid planting seedlings in the compacted upper soil, the compaction being caused by removal of cores from the corer. The cores were incubated in a heated glasshouse beneath mercury vapour lamps supplying 15 Wm^{-2} at plant level. The seedlings were harvested 9 weeks later and assessed for mycorrhizal infection; the oven-dry weights of the shoots were also then recorded.

A number of fruitbodies were seen on the plot late in October 1980. Their positions were marked and later a block of nine cores in a 3 x 3 arrangement was taken around the position of each fruitbody, the central core of the nine occupying the position of the fruitbody and the other cores being spaced 1 cm apart from rim to rim. All of these cores were dissected to assess the mycorrhizas in them.

Results from dissection cores

Considerable spatial variation in the numbers of mycorrhizas was found. For example, in no instance did all four cores taken at a single sampling distance from a tree contain mycorrhizas, and for one tree (W62) only 4 cores out of a possible 24 contained mycorrhizas (Table 3.2.2). No mycorrhizas were found in any of the control cores examined. Even on a small scale, the spatial variation in mycorrhizal numbers was quite marked, as shown in Figure 3.2.1, referring to the numbers of mycorrhizas in cores around fruitbodies. Although, overall, there was little relationship between the position of a fruitbody and the

TABLE 3.2.2: Numbers of soil cores (max. 4) containing mycorrhizas at six distances from each of four birch saplings.

Distance (cm)	Tree				Total (max. 16)
	W65	W62	Q68	N62	
10	3	3	3	3	12
20	3	0	1	2	6
35	1	1	2	1	5
50	3	0	1	0	4
65	2	0	1	1	4
80	2	0	1	0	3

TABLE 3.2.3: Mean numbers (± s.e.) of mycorrhizal tips found in cores taken at six distances from each of four birch saplings (n=4).

Distance (cm)	Tree				Total
	W65	W62	Q68	N62	
10	46 ± 22.6	20 ± 14.2	47 ± 16.0	109 ± 41.3	56 ± 15.8
20	40 ± 12.1	0	9 ± 5.0	5 ± 3.9	13 ± 5.4
35	4 ± 3.7	4 ± 4.0	9 ± 5.0	2 ± 1.9	5 ± 2.0
50	9 ± 4.6	0	10 ± 8.8	0	5 ± 2.9
65	1 ± 0.8	0	26 ± 22.5	2 ± 1.9	7 ± 6.5
80	4 ± 2.4	0	2 ± 1.5	0	1 ± 0.9

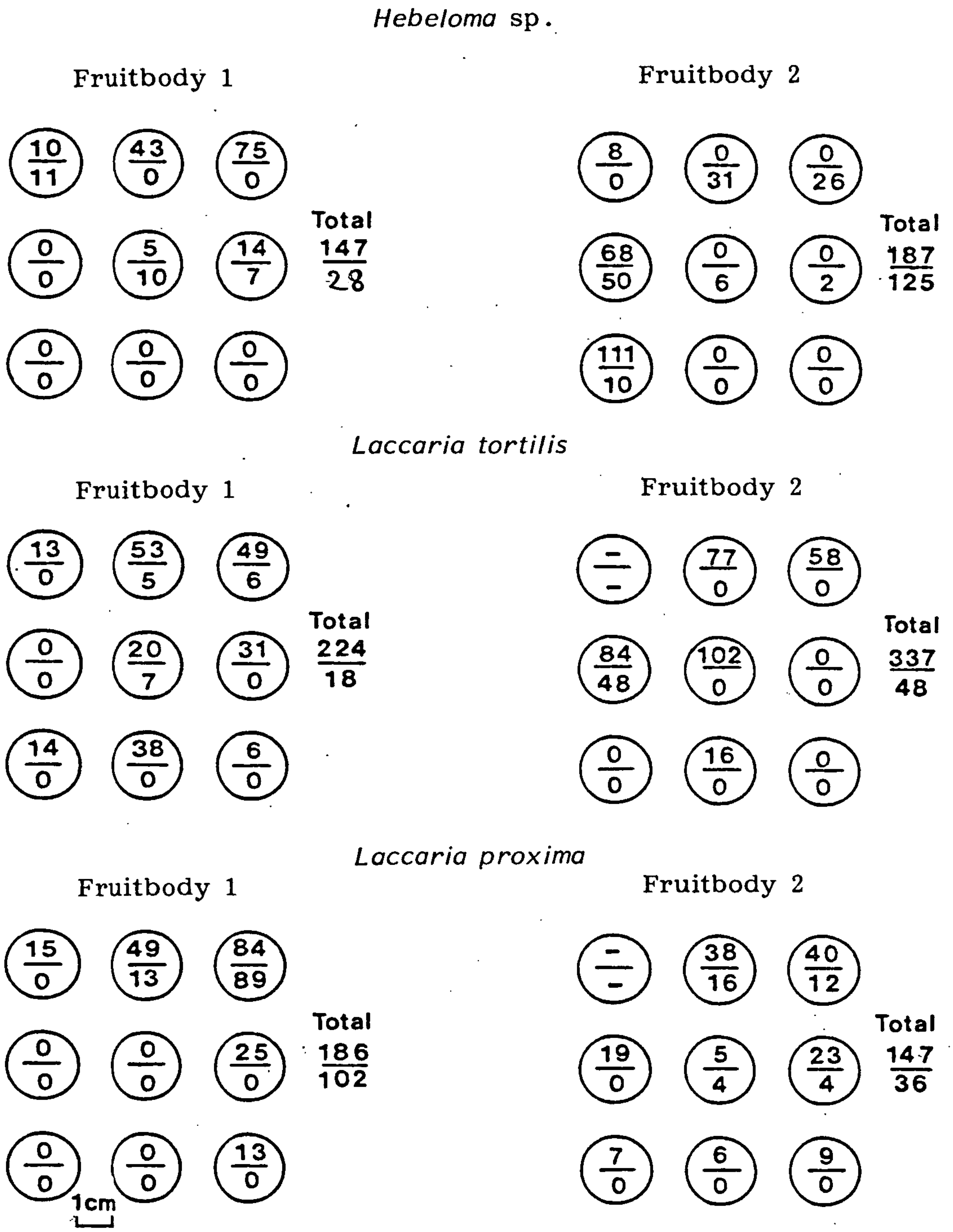

FIGURE 3.2.1: Numbers of mycorrhizas attributable to the fruiting species ($\frac{x}{0}$) and to other types ($\frac{0}{x}$) in soil cores taken from around and beneath a fungal fruitbody (the central core); the diagrams illustrate the relative positions of soil cores, one to another.

numbers of mycorrhizas directly beneath it, yet when the mycorrhizas for all nine cores were totalled the 'fruitbody'-type was always dominant (Figure 3.2.1). Furthermore, even when there were no, or few, mycorrhizas of the fruiting species present beneath the fruitbody there were also few mycorrhizas of other types present. However, only six fruitbodies were sampled in this way and it would be desirable to sample a greater number than this.

Most mycorrhizal tips observed in the samplings were found in cores taken at 10 cm distance from the trees; the numbers then fell off rapidly with distance (Table 3.2.3). But the trees differed, one from another, in this respect, because mycorrhizal tips could still be found at 80 cm distance from two of them (W65 and Q68) whereas no mycorrhizas were observed beyond 35 cm distance from one of the trees (W62).

The relationship between the presence of fruitbodies and mycorrhizas in the dissection cores is summarised in Table 3.2.5. It was not possible at the time of sampling to identify with certainty Inocybe-type mycorrhizas in the dissection cores, so, if present, any mycorrhizas formed by *Inocybe* spp. would have been included in the category termed 'others' (35.3% of all mycorrhizal tips) which could not be classified. Apparently dead mycorrhizal tips accounted for a further 28.7% of the total found, and Hebeloma- and Laccaria-types for 22.5% and 13.5% respectively. Excluding *Inocybe* sp. for reasons already given, inspection of Table 3.2.5 shows that mycorrhizas attributable to *Laccaria* sp. and *Hebeloma* sp. were always present on trees known to support fruitbodies of these fungi.

Results from seedling assays:

The results for seedlings grown in the soil cores contrast markedly in several respects with those from the dissection cores. For example, there was little difference in the numbers of mycorrhizas formed on the seedlings at different distances from the trees (Table 3.2.4). This is of interest because mycorrhizal establishment occurred on seedlings even in cores from sampling points where no mycorrhizas could be found by dissection. Nevertheless, in contrast to the case with 'test' cores taken along radii (Table 3.2.4), only 3 of 20 seedlings grown in 'control' cores had developed mycorrhizas, with a mean of 4 ± 2.5 mycorrhizal root tips and 34±4.2 uninfected root tips.

There was no significant correlation, overall, between percentage of root tips that were mycorrhizal and shoot dry weight of the seedlings, although there was such a correlation in the case of one tree, N62 ($r = 0.809$, $p = 0.001$). There was, however, a significant correlation, overall, between shoot dry weight and the total number of mycorrhizal root tips ($r = 0.536$, $p = 0.001$).

Recognition of Inocybe-type mycorrhizas was simpler on seedlings than by dissection of cores because the mycorrhizas were freshly formed; they accounted for 25.7% of the total number of mycorrhizal tips. Hebeloma-type mycorrhizas accounted for only 3.2% and Laccaria-type for 40.5% of the total on the seedling roots; four unknown types accounted for the remainder of mycorrhizal root tips on seedlings. It was not possible to distinguish between the mycorrhizas formed by different *Laccaria* species. Notwithstanding this, there was generally a close relationship between the recorded presence of fruitbodies of four mycorrhizal fungi and the occurrence of the respective mycorrhizal types assessed by a combination of dissection of soil cores and seedling assay

TABLE 3.2.4: Mean numbers (± s.e.) of mycorrhizal tips on 9-week-old birch seedlings grown in soil cores taken at five distances from each of four birch saplings (n=4).

Distance (cm)	Tree W65	W62	Q68	N62	Total
20	34 ± 14.9	15 ± 7.9	78 ± 14.2	23 ± 6.9	35 ± 15.7
35	12 ± 3.6	9 ± 5.5	30 ± 8.7	34 ± 12.2	21 ± 9.7
50	16 ± 7.1	23 ± 7.0	24 ± 11.8	12 ± 2.4	20 ± 8.3
65	32 ± 13.4	21 ± 7.2	36 ± 15.8	16 ± 4.9	26 ± 11.9
80	38 ± 4.1	8 ± 5.5	2 ± 0.8	12 ± 2.3	16 ± 8.1

TABLE 3.2.5: Summary of the occurrence of fruitbodies (F) in 1978-80, type of mycorrhizas in soil cores (M) in 1980 and type of mycorrhizas formed on seedlings (S) grown in soil cores from around each of four birch saplings.

Tree	*Hebeloma* sp.	*Laccaria tortilis*	*Laccaria proxima*	*Inocybe* sp.
W65	FM			FS
W62	M		FM	FS
N62	FMS	FMS*	FMS*	F
Q68			FMS	FS

*It was not possible to distinguish between the mycorrhizas of *L. tortilis* and *L. proxima*.

(Table 3.2.5). Of these assessments, the seedling assay seems to be less sensitive in demonstrating the presence of different mycorrhizal fungi than is dissection and direct observation, because the mycorrhizas of some fungi were observed in the cores but they did not become established on the seedlings.

3.3 The Effectiveness as Inoculum of the Mycorrhizal Roots of Birch

Excised mycorrhizal roots are reported to function as effective inocula for some mycorrhizal fungi; for example, Sinclair (1974) demonstrated that inoculation of 5-week-old Douglas-fir seedlings with fresh mycorrhizal roots resulted in increased numbers of mycorrhizas on seedlings 15 weeks later when compared to uninoculated plants. By contrast, Robertson (1954) found that detached roots of pine seedlings were not a good source of mycorrhizal inoculum compared with roots still attached to a living seedling. In view of the importance of this topic for experimental work on the inoculation of seedlings in soil, the following two experiments were done; it was hoped to see if soil type or the source of mycorrhizal root inoculum significantly affect the efficiency of mycorrhizal development on seedlings.

3.3.1 *Influence of Rooting Medium on the Effectiveness of Mycorrhizal Roots as a Source of Inoculum*

Rooting media for experimental purposes were collected from three locations in Midlothian and East Lothian, as follows.

1. Coal spoil from Roslin (OS reference NT 263634).
2. Agricultural brown earth from Boghall (OS reference NT 245653).
3. Fixed-dune sand from Gullane (OS reference NT 4682).

There were no trees within at least 10 m of any of the sampling points. Samples of mycorrhizal birch root systems were collected on 7 February 1981 from young birch trees growing on the coal spoil heap at Roslin. The roots were washed thoroughly under a running tap and then dried on filter paper. Woody roots were cut out and discarded so that only the fine mycorrhizal roots remained; the mycorrhizas were formed mainly by *Paxillus involutus* but included also a few attributable to *Laccaria* sp. The root material was thoroughly mixed and then divided into two lots, one of which was used as collected and the other was immersed in boiling water for 2 minutes. Then the roots were thoroughly mixed at 'single' and 'double' rates into the rooting media and the media were used to fill transparent polystyrene sample bottles (faeces bottles, code 128A, Sterilin Ltd), 8 cm deep and 2 cm internal diameter, with a drainage hole drilled in the bottom. The four treatments for each of the rooting media were as follows.

1. Addition of live roots, 0.1 g fresh weight per bottle.
2. Addition of live roots, 0.2 g fresh weight per bottle.
3. Addition of dead (boiled) roots, 0.1 g fresh weight per bottle.
4. No root supplement.

Each filled container, with its lid removed, was planted with an aseptically germinated birch seedling on 8 February 1981; the containers were then randomised in trays of moist sand, forty per tray, and covered with propagation lids. The trays were incubated in a heated glasshouse at about 20°C beneath 'Gro-lux' fluorescent strip lights, supplying 15 Wm^{-2} at plant level in an 18 h light/6 h dark cycle; the seedlings were watered as required.

On harvesting after 9 weeks (8 May 1981), the shoot dry weights and the numbers and types of mycorrhizal root tips and numbers of uninfected root tips were recorded (Tables 3.3.1 and 3.3.2). When the results for all the inoculation treatments are pooled, the seedlings grown in brown earth are seen to have been significantly heavier than the seedlings grown in the two other rooting media (Table 3.3.1); this result was reflected in all the inoculation treatments. Similarly, when the results for all types of rooting medium are pooled, seedlings grown in the presence of 'live' inoculum were significantly heavier than those that had been grown without inoculum or with heat-killed root pieces. However, when the results for each rooting medium are considered separately, only in coal spoil was there a significant effect of live inoculum on the shoot growth of seedlings (Table 3.3.2).

On inspection of the seedling root systems (Table 3.3.3) very few mycorrhizas had developed in the brown earth or dune sand whereas large numbers of mycorrhizas had developed on seedlings in coal spoil; these mycorrhizas were formed predominantly by *P. involutus*, to which fungus 60% of all the mycorrhizas in coal spoil were attributable. Other, less common, mycorrhizas in the coal spoil were attributable to *Laccaria* sp. or *Inocybe* sp., and the few mycorrhizas observed on seedlings in the brown earth or dune sand were formed by *Hebeloma* sp.

In the coal spoil (Table 3.3.4), but not in the other media, there was a significant effect of inoculation on the numbers of mycorrhizas formed on seedlings. Thus, both the single and double 'live inoculum' treatments resulted in a significant enhancement of mycorrhizal development compared with in the 'dead inoculum' and control treatments, even though there was no difference in the total numbers of root tips between the treatments. Across the treatments in coal spoil, there was a highly

TABLE 3.3.1: Mean shoot oven-dry weights (mg) of seedlings grown for 9 weeks in three different rooting media (pooled results for inoculation treatments) or in rooting media supplemented with different mycorrhizal inocula (pooled results for rooting media); data transformed to log(x+1) for analysis (in parentheses).

Rooting medium			L.S.D.
Brown earth	Dune sand	Coal spoil	(p=0.05)
43.8	2.7	4.5	
(1.608)	*(0.533)*	*(0.687)*	*(0.087)*

Inoculum addition*				
Live roots (double)	Live roots	Dead roots	None	
17.5	18.5	16.0	16.0	
(1.000)	*(1.008)*	*(0.883)*	*(0.879)*	*(0.100)*

*For explanation, see text.

TABLE 3.3.2: Mean shoot oven-dry weights (mg) of birch seedlings grown for 9 weeks in three rooting media supplemented with different mycorrhizal inocula; data transformed to log(x+1) for analysis (in parentheses).

	Inoculum addition				
Rooting medium	Live roots (double)	Live roots	Dead roots	None	L.S.D. (p=0.05)
Brown earth	43.4 *(1.616)*	46.7 *(1.655)*	42.7 *(1.579)*	42.7 *(1.585)*	*(0.210)*
Dune sand	2.5 *(0.530)*	3.2 *(0.577)*	2.4 *(0.504)*	2.5 *(0.521)*	*(0.150)*
Coal spoil	6.6 *(0.854)*	5.6 *(0.791)*	2.9 *(0.567)*	2.7 *(0.534)*	*(0.161)*

TABLE 3.3.3: Mean numbers (± s.e.) of root tips on birch seedlings grown for 9 weeks in three different rooting media; results for different inoculation treatments pooled (n=40).

Rooting medium	Mycorrhizal root tips	Total root tips
Brown earth	0 ± 0.0	182 ± 13.6
Dune sand	3 ± 1.7	203 ± 132.2
Coal spoil	43 ± 5.3	77 ± 4.6

TABLE 3.3.4: Mean numbers of root tips and mycorrhizal root tips, and mean percentage of root tips occupied by mycorrhizas, on seedlings grown for 9 weeks in coal spoil supplemented with four mycorrhizal inoculum additions; data transformed to log(x+1) or to angles for analysis (in parentheses).

	Inoculum addition				
	Live roots (double)	Live roots	Dead roots	None	L.S.D. (p=0.05)
Mycorrhizal root tips (log x+1)	65 *(1.794)*	61 *(1.750)*	20 *(0.778)*	24 *(0.957)*	*(0.521)*
Total root tips (log x+1)	90 *(1.943)*	76 *(1.862)*	72 *(1.806)*	70 *(1.802)*	*(0.182)*
% roots mycorrhizal (angles)	71.3 *(58.0)*	77.1 *(62.6)*	20.1 *(19.9)*	27.2 *(26.2)*	*(15.4)*

significant correlation between the numbers of mycorrhizal tips and shoot dry weight (r = 0.801, p = 0.001).

This experiment therefore demonstrates that excised mycorrhizal roots can function as inoculum for the colonisation of seedling roots, but it is notable that such inoculum was successful only in coal spoil, possibly because the inoculum came originally from this source. Also of interest is the fact that successful mycorrhizal inoculation in this rooting medium resulted in significantly enhanced shoot dry weight.

3.3.2 *The Effectiveness of Mycorrhizal Roots from Three Contrasting Sites as Inoculum in Coal Spoil*

In the experiment just described, it was shown that mycorrhizal roots collected from a coal spoil functioned as effective inoculum only in the coal spoil itself. An experiment was therefore now done to see if mycorrhizal roots from other sources were as effective as those from coal spoil when added as inoculum to coal spoil.

Mycorrhizal roots were collected on 21 June 1981 from birch trees growing on a coal spoil heap at Roslin, on a raised peat bog at Threipmuir (OS reference 164368) and in a mature mixed woodland at Penicuik (OS reference 218605). The roots were washed and dried as described earlier. 'Non-mycorrhizal' coal spoil was collected at Roslin from a point at least 10 m distance from any trees. The soil containers were prepared as described in Section 3.3.1, but there were only two treatments for each inoculum source: (1) addition of 0.1 g fresh weight of live roots; (2) addition of 0.1 g boiled roots. A third treatment consisted of no inoculum addition. Fifteen replicates of each of the seven treatments (3 inoculum sources x 2 inoculum treatments + control) were prepared and planted with aseptically germinated birch seedlings on 22 June 1981. The containers were randomised and stood in moist sand

TABLE 3.3.5: Mean shoot oven-dry weights (mg) of seedlings grown for 79 days in coal spoil supplemented with inoculum from three sites; data transformed to log(x+1) for analysis (in parentheses).*

Source of inoculum						
Roslin		Threipmuir		Penicuik		
live	dead	live	dead	live	dead	None
8.52	5.81	11.64	7.16	7.52	12.22	9.84
(0.960)	*(0.756)*	*(1.092)*	*(0.857)*	*(0.857)*	*(1.070)*	*(1.029)*

*there was no significant difference between the treatments when analysed by the Student-Newman-Keuls test.

TABLE 3.3.6: Mean numbers of mycorrhizal tips on seedlings grown for 79 days in coal spoil supplemented with inoculum from three sites; data transformed to log(x+1) for analysis (in parentheses).*

Source of inoculum						
Roslin		Threipmuir		Penicuik		
live	dead	live	dead	live	dead	None
106.9	71.1	140.8	103.5	96.8	126.4	112.1
(2.000)	*(1.602)*	*(2.121)*	*(1.901)*	*(1.698)*	*(2.020)*	*(2.008)*

* there was no significant difference between the treatments when analysed by the Student-Newman-Keuls test.

as before, covered with propagator lids, but incubated in a growth cabinet (Section 2.2.3). Shoot dry weights and mycorrhizal development on the roots were assessed on 9 September 1981, 79 days after sowing (Table 3.3.5).

Several seedlings in all treatments had died, and those that remained showed no significant difference in either shoot dry weight or the mean numbers of mycorrhizal tips (Tables 3.3.5 and 3.3.6). Overall, 81.1% of all mycorrhizas were attributable to *Paxillus involutus* irrespective of treatment; the high level of colonisation by this mycorrhizal fungus must be ascribed to the presence of sufficient naturally occurring inoculum in the coal spoil. The experiment, therefore, gave inconclusive results and it was not repeated.

3.4 Mycorrhizal Development on Birch Seedlings Grown in Soils Previously Planted with Other Tree Species

Little is known about the survival of mycorrhizal fungi in the absence of a suitable host, although it has been reported that numbers of mycorrhizal propagules in soil may be depleted by continuous application of agricultural or nursery practices (Trappe and Strand, 1969). This experiment was done to see if seedlings of several different tree species could establish mycorrhizas which could then provide inoculum for colonisation of birch seedlings. The tree species were chosen such that two - birch and Sitka spruce (*Picea sitchensis*) - typically bear sheathing mycorrhizas and one, sycamore (*Acer pseudoplatanus* L.), forms only endomycorrhizas.

Mycorrhizal soil was collected on 21 March 1982 from beneath a stand of 10-year-old birch trees growing on the Bush Estate. The soil was mixed thoroughly, to ensure an even distribution of inoculum, and then distributed between forty-eight open transparent polystyrene bottles, 8 cm deep and 2 cm in diameter. On the following day, 22 March, twelve of the containers were planted with a single pre-germinated birch seedling, twelve with a Sitka spruce seedling and twelve with a sycamore seedling, the remaining twelve containers being left as controls. The containers were randomised in trays of moist sand and incubated in a growth room for 93 days. The seedling shoots were then cut off at soil level (23 June 1982) and their heights and oven-dry weights were recorded. Three each of the birch and Sitka spruce seedling root systems were sampled and assessed for mycorrhizas; all the remaining containers were then planted with a pre-germinated birch seedling and re-incubated in the growth room. On 3 November 1982, after 102 days' growth, the birch assay seedlings were sampled (Tables 3.4.3 and 3.4.4).

At the end of the initial planting, the sycamore seedlings were much taller and heavier than the birch and spruce seedlings (Table 3.4.1) and can therefore be expected more thoroughly to have depleted the soil of mineral nutrients; the birch seedlings were heavier than the spruce but not to the same degree as in the case of sycamore. Of interest, the birch seedlings developed a wider range of mycorrhizal types than did Sitka spruce (Table 3.4.2), and the mycorrhizas of *Thelephora terrestris* were both numerically and proportionately much more common on birch than Sitka spruce. Although there were, in total, a greater number of root tips on the birch seedlings than on the Sitka spruce, this can be attributed to different root morphologies rather than to differences in growth.

The birch seedlings grown as the second (assay) crop showed no significant difference in height between any of the treatments (Table

TABLE 3.4.1: Mean (± s.e.) shoot height and oven-dry weight of sycamore, birch and Sitka spruce seedlings grown for 93 days in mycorrhizal soil from beneath the canopy of birch.

	Sycamore (n=12)	Birch (n=12)	Sitka spruce (n=11)
Height (mm)	79 ± 6.0	35 ± 3.0	35 ± 4.4
Dry weight (mg)	178 ± 16.7	21 ± 2.8	11 ± 1.3

TABLE 3.4.2: Mean numbers (± s.e.) of root tips of different mycorrhizal types on birch and Sitka spruce seedlings grown for 93 days in 'mycorrhizal' soil from beneath the canopy of birch (n=3).

Mycorrhizal type	Birch	Sitka spruce
Hebeloma	29 ± 7.3	15 ± 2.1
Thelephora	47 ± 6.9	3 ± 2.7
Others	13 ± 8.1	0
Uninfected	27 ± 6.3	8 ± 3.0
Total root tips	115 ± 12.2	26 ± 4.8

TABLE 3.4.3: Mean shoot height and oven-dry weight of birch seedlings grown for 102 days in soil that had previously borne seedlings of sycamore, birch or Sitka spruce, or no seedlings; data transformed to log(x+1) for analysis (in parentheses).

	Former tree species			
	Sycamore	Birch	Sitka spruce	None
Height (mm)	28.7 *(1.460)*	29.0 *(1.471)*	33.5 *(1.531)*	35.9 *(1.552)*
Dry weight (mg)	20.4^{a} *(1.277)*	23.2^{a} *(1.342)*	36.1^{a} *(1.559)*	34.5^{a} *(1.475)*

* Figures followed by the same letter differ from one another at p=0.01.

TABLE 3.4.4: Mean numbers of root tips on birch seedlings grown for 102 days in soil that had previously borne sycamore, birch, Sitka spruce or no seedlings; data transformed to log(x+1) for analysis (in parentheses).*

	Former tree species			
	Sycamore	Birch	Sitka spruce	None
Mycorrhizal root tips	39^{ab} *(1.563)*	91^{a} *(1.906)*	116^{ab} *(2.019)*	83^{b} *(1.844)*
Uninfected root tips	23 *(1.256)*	19 *(1.100)*	31 *(1.445)*	33 *(1.399)*
Total root tips	62^{ab} *(1.759)*	110^{a} *(2.014)*	147^{ab} *(2.159)*	116^{b} *(2.019)*

* Figures in each row followed by the same letter differ from one another at p = 0.01.

TABLE 3.4.5: Mean percentage of root tips occupied by different mycorrhizal types on birch seedlings grown for 102 days in soil that had previously borne sycamore, birch, Sitka spruce or no seedlings; data transformed to angles for analysis (in parentheses).*

Mycorrhizal type	Former tree species			
	Sycamore	Birch	Sitka spruce	None
Hebeloma	13.0 *(12.8)*	27.7 *(24.9)*	30.7 *(28.5)*	25.5 *(26.3)*
Type 15	46.4 *(41.4)*	26.5 *(25.4)*	24.0 *(23.9)*	31.8 *(32.2)*
Others	4.8 *(4.1)*	23.5 *(22.3)*	20.1 *(21.1)*	7.7 *(10.1)*
Total mycorrhizal root tips	62.6 *(54.2)*	78.6 *(61.5)*	83.0 *(66.9)*	71.2 *(56.5)*

* There were no significant differences between the treatments when the results were analysed by the Student-Newman-Keuls test.

3.4.3), but they were heaviest in the containers (controls and with pre-planted Sitka spruce) that had supported least growth of the first-sown seedlings (c.f. Tables 3.4.1 - 3.4.3). This difference was significant when analysed by the Student Newman-Keuls test, a 'multiple-range' test that takes account of different numbers of replicates.

Interpretation of the effects of previous 'cropping' on the mycorrhizal status of the birch assay crop (Tables 3.4.4 and 3.4.5) is, unfortunately, complicated by several potentially interacting factors. For example, not surprisingly, the smallest birch seedlings - those grown in soil that had previously supported sycamore - also had the smallest numbers of roots and thus the fewest mycorrhizas (Table 3.4.4). But the percentage of the root tips that were mycorrhizal in this treatment was nearly as high as in the other treatments (Table 3.4.5) and did not differ significantly in this respect. Similarly, there was no significant difference in infection by any mycorrhizal type between the treatments and, in this respect, it can be seen that the assay seedlings grown in soil that had previously borne birch or Sitka spruce had root systems occupied by a similar range and proportion of types. It seems, therefore, that growing different tree species in the soils over the time period of the experiment had little direct effect on the relative inoculum levels of the different mycorrhizal fungi. It is likely that different degrees of mineral nutrient depletion in the different treatments account for the major differences in growth of the assay seedlings.

3.5 Discussion

The experiments in this section provide some information on the distributions of mycorrhizas around the bases of trees, on the appropriateness of sampling techniques to determine mycorrhizal distributions and on some of the factors that may influence the effectiveness of different mycorrhizal inocula.

In Section 3.2, the distribution of mycorrhizas around four 5-year-old birch saplings was examined. Although the majority of mycorrhizal tips were found at 10 cm distance from the tree (Table 3.2.3), overall there was considerable variation in mycorrhizal numbers. It was not clear if this was because of a poor sampling procedure or whether the distribution of mycorrhizas was actually very variable. It is unlikely that in the area of soil sampled there was complete root exploration by the young trees. Reference to the plans of excavated birch root systems made by Laitakari (1935) illustrate that even with mature root systems there is not a uniform distribution of roots. Ford *et al.* (1980) found distinct arcs around birch trees that were not occupied by any sporophores of mycorrhizal fungi. If these represent areas with no birch roots or mycorrhizas then the position of a transect or radius from the tree may markedly affect the results obtained. This is acceptable if enough transects are used to give an accurate portrayal of the root system, but whether or not this was achieved in the present experiments is unknown. Weller (1971) used ten radiating transects and 4 cm diameter soil cores to study the distribution of the absorbing roots of fruit trees. A similar sampling procedure was adopted in subsequent sections of this thesis, whenever appropriate, by using a greater number of transects or soil cores or both.

A high proportion (28.7%) of dead root tips was observed in the cores taken from around the four birch saplings (Section 3.2), probably because the soil cores were collected in October when a proportion of dead root tips may be expected. Harvey, Jurgensen and Larsen (1978) found that 95% of all 'active' mycorrhizal tips in a Douglas-fir/larch forest in Montana occurred between the months of May and October, with 65% between May and June. It is possible that there is a similar pattern for birch; if so, then sampling in early summer may result in greater numbers and relative proportions of 'active' mycorrhizas being found. Even so, it is likely that mycorrhizas sampled in the winter accurately reflect the proportions and distributions of mycorrhizas formed in the previous growing season, provided that sampling is done before new root growth occurs, when new mycorrhizas begin to form.

Much more mycorrhizal infection was observed when seedlings were grown in soil cores taken from around four birch saplings than when the cores themselves were examined (c.f. Tables 3.2.3 and 3.2.4); interestingly, the seedlings developed mycorrhizas even in cores taken where no mycorrhizas could be found in the soil. This indicates that infection occurred from inoculum other than mycorrhizal roots - possibly from spore inoculum (Robertson, 1954). Such inoculum would be present in the soil around the trees, especially in autumn when the cores were collected, because most fruitbodies of mycorrhizal basidiomycetes are produced in autumn.

Although spores would seem to be the likely inoculum for the mycorrhizal colonisation of seedling assays (Section 3.2), yet excised mycorrhizal roots were demonstrated to be an effective type of inoculum, especially of *Paxillus involutus*, in coal spoil (Table 3.3.4). However, the mycorrhizal roots used as inoculum in this experiment did not colonise seedlings grown in two other contrasting rooting media (Table 3.3.3); that is, the inoculum was only successful in the medium from which it was initially gathered. *P. involutus* is frequently recorded from coal spoil heaps, as both fruitbodies and mycorrhizas, but is less frequent on other types of sites; thus field observations are supported by evidence from this experiment. So whilst this experiment supports the results of Sinclair (1974), that excised mycorrhizal roots may function as effective inoculum, it also shows that edaphic factors may influence mycorrhizal colonisation of seedlings from such inoculum and that this should be borne in mind when interpreting the results from other experiments. It is unclear why, in a subsequent experiment (Section 3.3.2), addition of inoculum to the same coal spoil had no effect on the mycorrhizal colonisation of the seedlings. It may be that the spoil collected had a high level of resident inoculum, or that there may have been a degree of 'cross-contamination' between the treatments. In any event, it is not possible from the experiment to determine whether mycorrhizal roots from sources other than coal spoil can function as inoculum in coal spoil.

Interestingly, the only experiment in which seedling growth was positively related to mycorrhizal infection concerned the use of coal spoil (Section 3.3.1): supplementing the spoil with live mycorrhizal roots resulted in a greater degree of mycorrhizal infection, but not a greater number of root tips, and in correspondingly larger shoots (c.f. Tables 3.3.2 and 3.3.4). In other experiments, the shoot growth of the seedlings must have been limited by factors other than mycorrhizal infection. For example, in Section 3.4 the growth of birch seedlings was inversely related to the growth of the seedlings formerly grown in the same soil (c.f. Tables 3.4.1 and 3.4.3). It seems, therefore, that growth of the seedlings in the second 'crop' was limited by the degree of nutrient depletion caused by growth of the first 'crop'. From this experiment, it would also seem that the length of time during which other tree species were grown was insufficient to have any effect on the resident mycorrhizal inoculum but, as was stated earlier, there were several difficulties of interpretation with this experiment. It is possible that spores were responsible for the mycorrhizal colonisation of seedlings grown in the soil previously planted with other tree seedlings; otherwise, it is difficult to explain the relatively high degree of mycorrhizal development on birch seedlings grown after sycamore - an endomycorrhizal tree.

CHAPTER IV

Factors Associated with Establishment, Persistence and Replacement of Mycorrhizal Fungi on Seedling Roots in Glasshouse and Field Conditions

4.1 Introduction

The distinction between 'early-stage' and 'late-stage' mycorrhizal fungi made by Deacon *et al*. (1983) was based on the ability of the fungi to infect seedlings from inoculum in soil cores or added to unsterile soil: only 'early-stage' fungi infect in these conditions. However, the evidence to date does not preclude several possibilities - for example, that 'late stage' mycorrhizal fungi might develop on prolonged incubation of seedlings or that they might have an obligatory requirement for seedlings to be infected by 'early-stage' fungi before they, in turn, can infect. These possibilities were investigated in this section.

The terms 'early-stage' and 'late-stage', as defined by Deacon *et al*. (1983), refer only to establishment of infection from inoculum in isolated volumes of soil and do not necessarily relate to persistence of mycorrhizal associations once established. There are many reports of the failure of established mycorrhizal fungi to persist on, and spread with, growing roots after outplanting of tree seedlings (Lamb, 1979; Bledsoe *et al*., 1982; Grossnickle and Reid, 1982); although little is known about the factors associated with this, Marks and Foster (1967) suggested that an interruption to root growth may be necessary. Some of the experiments in this section were designed to investigate the persistence of established mycorrhizal symbioses, with particular regard to the behaviour of 'early-stage' and 'late-stage' fungi.

4.2 Factors Affecting the Formation of Mycorrhizas by Some 'Late-Stage' Mycorrhizal Fungi

Deacon *et al*. (1983) reported that 'late-stage' mycorrhizal fungi, such as *Lactarius pubescens*, *Leccinum* spp. and *Amanita muscaria*, do not colonise seedling roots from inoculum added to soil in which the seedlings are grown, although these fungi readily form mycorrhizas on seedlings in aseptic conditions. The two experiments described in this section were done to investigate some of the factors that may influence the formation of mycorrhizas by such 'late-stage' fungi.

4.2.1 *The Effect of Inoculating Birch Seedlings with 'Early-Stage' Mycorrhizal Fungi on the Formation of Mycorrhizas by 'Late-Stage' Fungi from Soil-borne Inoculum*

The fruitbodies and mycorrhizas of 'late-stage' mycorrhizal fungi, *sensu* Deacon *et al.* (1983), tend to occupy positions around trees that have formerly been occupied by 'early-stage' fungi (Mason *et al.*, 1982; Deacon *et al.*, 1983). It was thus considered possible that colonisation of a root system by 'late-stage' fungi may be facilitated in some way by the presence of a previously established 'early-stage' fungus. Alternatively, the established mycorrhizas of 'early-stage' fungi may prevent or delay colonisation of root systems by other mycorrhizal fungi - a possibility that would be consistent with the experiments of Deacon *et al.* (1983) because such 'early-stage' mycorrhizal types consistently develop on seedlings in soil, even during relatively short incubation times.

This experiment was done to see if two fungi, *Hebeloma sacchariolens* and *Laccaria proxima* (Boud.) Pat., pre-established on seedling roots affected the formation of mycorrhizas of 'late-stage' fungi when the seedlings are transplanted into soil supplemented with inoculum of such fungi. In addition, the experiment was designed to assess the abilities of the two 'early-stage' fungi to colonise, and persist on, an actively growing root system transplanted into fresh soil.

Method:

Aseptically germinated birch seedlings were transferred, on 3 April 1981, to propagators (Section 2.3.2) containing sterilised vermiculite-peat which was either uninoculated (and therefore sterile) or inoculated with *H. sacchariolens* (isolate 4) or *L. proxima* (isolate 4); the propagators were then incubated in growth cabinets. 'Non-mycorrhizal' Bush soil was collected on 18 May 1981 and, after being passed through a 1 cm² sieve, was mixed (2 : 1 v/v) with either sterile vermiculite-peat or vermiculite-peat inoculum of *Lactarius pubescens* (isolate 6), *Leccinum scabrum* (Fr.) S.F.Gray (isolate 6) or *Amanita muscaria* (isolate 71) prepared as described in Section 2.1.3. The initial pH of the soil was 6.4, but this fell to pH 5.4 after adding the inoculum. Care was taken to prevent cross-contamination between the variously supplemented soils during mixing, and the soils were then added to clean 75 mm diameter plastic pots. Twelve replicate randomised blocks were prepared, each containing factorial combinations of the three seedling inoculation treatments and the four soil treatments. The seedlings were transplanted to the soils on 20 May 1981, 47 days old, after having removed the plastic sleeves in which they had grown, and the pots were then incubated in a heated glasshouse (maximum temperature 31°C, minimum 9.5°C). All seedlings in the pre-inoculation treatments are known to have been mycorrhizal when transplanted.

Three blocks were selected randomly for sampling after a further 47 days (6 July 1981) and three after 117 days (14 September); the remaining six blocks were then re-potted into 127 mm square pots containing unsupplemented 'non-mycorrhizal' Bush soil. Three more blocks were sampled after 190 days (25 November). A number of replicates had been lost by this time because of an infestation of the soil by the larvae of *Otiorhynchus* sp.; to control this the remaining pots received, on 10 December, a soil drench of 'Ambush' insecticide. On 14 December the plants were transferred to a growth chamber with a daily 8-hour photoperiod supplied by three fluorescent 'Gro-lux' strip lights (light intensity at plant level 15 Wm^{-2}; temperature 15-20°C) to induce dormancy. After 73 days in this environment (25 February 1982) and while still leafless, the plants were transferred to an unheated but frost-free glasshouse with no supplementary lighting. The plants remained in this glasshouse until 3 August 1982, 441 days after initial transplanting into inoculum-supplemented soil, when they were sampled.

At each sampling the height and oven-dry weight of the shoots was recorded; the root systems were cut into 5 cm long pieces from the base of the stem, as described in Section 2.5.2c, and assessed for mycorrhizal infection. Root sections with very high numbers of root tips were, when necessary, subsampled (Section 2.5.2c). Fungal fruit-bodies appearing in the pots were recorded as they occurred.

Results:

At the time of transplanting, the non-mycorrhizal control seedlings were significantly taller and heavier than their mycorrhizal counterparts (Table 4.2.1). The control seedlings still had significantly heavier and taller shoots than did the inoculated seedlings 47 days after transplanting and in some cases 117 days after transplanting, but the differences in shoot dry weight were no longer significant at 190 days. At the end of the experiment, 441 days after transplanting, there was no significant difference in shoot growth between treatments, though the plants inoculated with *L. proxima* were slightly smaller than the others. Table 4.2.3 shows the pooled results for different propagation treatments and thus enables overall effects of soil supplementation on shoot growth

TABLE 4.2.1: Mean shoot height and oven-dry weight of seedlings incubated for 47 days in sterile vermiculite-peat or in vermiculite-peat inoculum of *Hebeloma sacchariolens* and *Laccaria proxima*; data transformed to log(x+1) for analysis (in parentheses).

	Inoculant fungus			L.S.D.
	H. sacchariolens	*L. proxima*	None	(p = 0.05)
Height (mm)	13 *(1.149)*	12 *(1.100)*	21 *(1.385)*	*(0.145)*
Dry weight (mg)	1.25 *(0.343)*	1.45 *(0.384)*	3.98 *(0.685)*	*(0.083)*

TABLE 4.2.2: Mean shoot heights and oven-dry weights of seedlings grown in variously supplemented soil after propagation in sterile vermiculite-peat or in vermiculite-peat inoculum of *Hebeloma sacchariolens* or *Laccaria proxima*; data pooled for different soil supplements and transformed to log(x+1) for analysis (in parentheses).

Days after transplanting	No. of seedlings sampled	Inoculant fungus *H. sacchariolens*	 *L. proxima*	 None	L.S.D. (p = 0.05)
Heights (mm)					
47	12	21 *(1.343)*	22 *(1.344)*	33 *(1.523)*	*(0.065)*
117	12	52 *(1.723)*	55 *(1.743)*	67 *(1.826)*	*(0.059)*
190	8	188 *(2.261)*	159 *(2.186)*	217 *(2.336)*	*(0.135)*
441	8	380 *(2.573)*	312 *(2.482)*	398 *(2.590)*	*(0.119)*
Dry weights (mg)					
47	12	12.7 *(1.079)*	15.9 *(1.083)*	30.9 *(1.453)*	*(0.195)*
117	12	93.4 *(2.001)*	120.8 *(2.080)*	143.0 *(2.150)*	*(0.078)*
190	8	786.1 *(2.884)*	771.9 *(2.878)*	802.3 *(2.903)*	*(0.122)*
441	8	3124.2 *(3.486)*	2904.0 *(3.439)*	3538.2 *(3.523)*	*(0.148)*

TABLE 4.2.3: Mean shoot heights and oven-dry weights of seedlings grown in variously supplemented soil after propagation in sterile vermiculite-peat or in vermiculite-peat inoculum of *Hebeloma sacchariolens* or *Laccaria proxima*; data pooled for different propagation treatments and transformed to log(x+1) for analysis (in parentheses).

Days after transplanting	No. of seedlings examined	Soil inoculum addition None	*Lactarius pubescens*	*Leccinum scabrum*	*Amanita muscaria*	L.S.D. (p= 0.05)
Heights (mm)						
47	9	21 *(1.330)*	29 *(1.463)*	24 *(1.399)*	26 *(1.420)*	*(0.075)*
117	9	56 *(1.747)*	59 *(1.769)*	57 *(1.760)*	60 *(1.770)*	*(0.069)*
190	6	191 *(2.260)*	198 *(2.288)*	180 *(2.245)*	183 *(2.251)*	*(0.154)*
441	6	396 *(2.588)*	349 *(2.532)*	355 *(2.545)*	354 *(2.530)*	*(0.138)*
Dry weights (mg)						
47	9	14.6 *(1.125)*	28.5 *(1.423)*	10.2 *(0.922)*	26.0 *(1.353)*	*(0.226)*
117	9	119.2 *(2.060)*	123.9 *(2.086)*	123.4 *(2.083)*	109.8 *(2.079)*	*(0.090)*
190	6	801.6 *(2.889)*	814.3 *(2.907)*	783.7 *(2.889)*	747.5 *(2.868)*	*(0.141)*
441	6	3117.8 *(3.473)*	3057.7 *(3.471)*	3038.2 *(3.452)*	3541.7 *(3.537)*	*(0.243)*

to be seen. After 47 days, seedlings grown in the soil supplemented with *L. pubescens* or *A. muscaria* were taller and heavier than the seedlings grown in unsupplemented soil or in soil supplemented with *L. scabrum*. However, these differences did not persist after the first sampling time.

All of the inoculated seedlings examined at the time of transplanting bore mycorrhizas of the inoculant fungi, whereas there were no mycorrhizas on the control seedlings (Table 4.2.4). Indeed, no mycorrhizas had formed on the control seedlings 47 days later and, at this stage, all of the mycorrhizas on the inoculated seedlings were attributable to the inoculant fungi. By 117 days, mycorrhizas formed from naturally occurring inoculum had developed on all the seedlings and were present in increasing numbers at subsequent sampling dates; these mycorrhizas were mainly attributable to *Hebeloma* sp., *Thelephora terrestris* and *Laccaria* sp. There were, however, substantial differences between treatments in this respect: 'other' mycorrhizal types were significantly more common on controls (originally non-mycorrhizal seedlings) than on seedlings inoculated with *H. sacchariolens* or *L. proxima*, while all treatments showed similar numbers of uninfected root tips. Another important and related feature of the results was the progressive increase in numbers of mycorrhizas (though not necessarily in the percentage of root tips colonised) attributable to the inoculant fungi; clearly both *H. sacchariolens* and *L. proxima* were able progressively to colonise the seedling roots in soil, to the partial exclusion of 'resident' or 'contaminant' mycorrhizal fungi which colonised roots of the control (originally non-mycorrhizal) seedlings in large numbers.

The records for spatial distributions of mycorrhizal types on the root system (Figures 4.2.1, 4.2.2 and 4.2.3) largely support the

TABLE 4.2.4: Occurrence of mycorrhizas on seedlings propagated in sterile vermiculite-peat or in vermiculite-peat inoculum of *Hebeloma sacchariolens* and *Laccaria proxima* and then transplanted into soil supplemented with inocula of different mycorrhizal fungi; data pooled for soil supplementation treatments.

Inoculant fungus (on seedlings)	Sampling time (days after transplanting)	No. of seedlings	Number of root tips (mean ± s.e.) with inoculant type mycorrhizas	with other mycorrhizal types	uninfected	total	No. of sclerotium-like bodies of *H. sacchariolens*
Hebeloma sacchariolens	0		24 ± 3.2	0	9 ± 2.0	33 ± 5.0	26 ± 3.3
	47	12	43 ± 6.7	0	65 ± 11.6	108 ± 16.6	28 ± 8.5
	117	12	570 ± 64.6	34 ± 26.1	126 ± 9.2	730 ± 68.7	793 ± 77.9
	190	11	920 ± 69.3	218 ± 142.8	251 ± 23.0	1390 ± 100.6	785 ± 89.0
	441	11	4125 ± 864.3	1966 ± 733.9	489 ± 106.5	6581 ± 685.2	4505 ± 601.5
Laccaria proxima	0		4 ± 2.1	0	25 ± 4.7	29 ± 2.7	0
	47	12	101 ± 25.2	0	122 ± 15.0	223 ± 30.8	0
	117	12	398 ± 100.9	359 ± 72.5	176 ± 17.1	933 ± 103.1	0
	190	12	848 ± 95.2	72 ± 20.2	348 ± 36.1	1268 ± 77.6	0
	441	10	7224 ± 1112.9	2084 ± 996.1	451 ± 55.1	9759 ± 1007.5	213 ± 213.1
None	0		-	0	49 ± 5.5	49 ± 5.5	0
	47	12	-	0	581 ± 85.9	581 ± 85.7	0
	117	12	-	867 ± 71.6	128 ± 6.8	995 ± 69.6	3 ± 3.3
	190	10	-	1462 ± 155.0	360 ± 35.0	1822 ± 134.0	0
	441	12	-	9488 ± 1014.4	220 ± 38.9	10441 ± 167.1	0

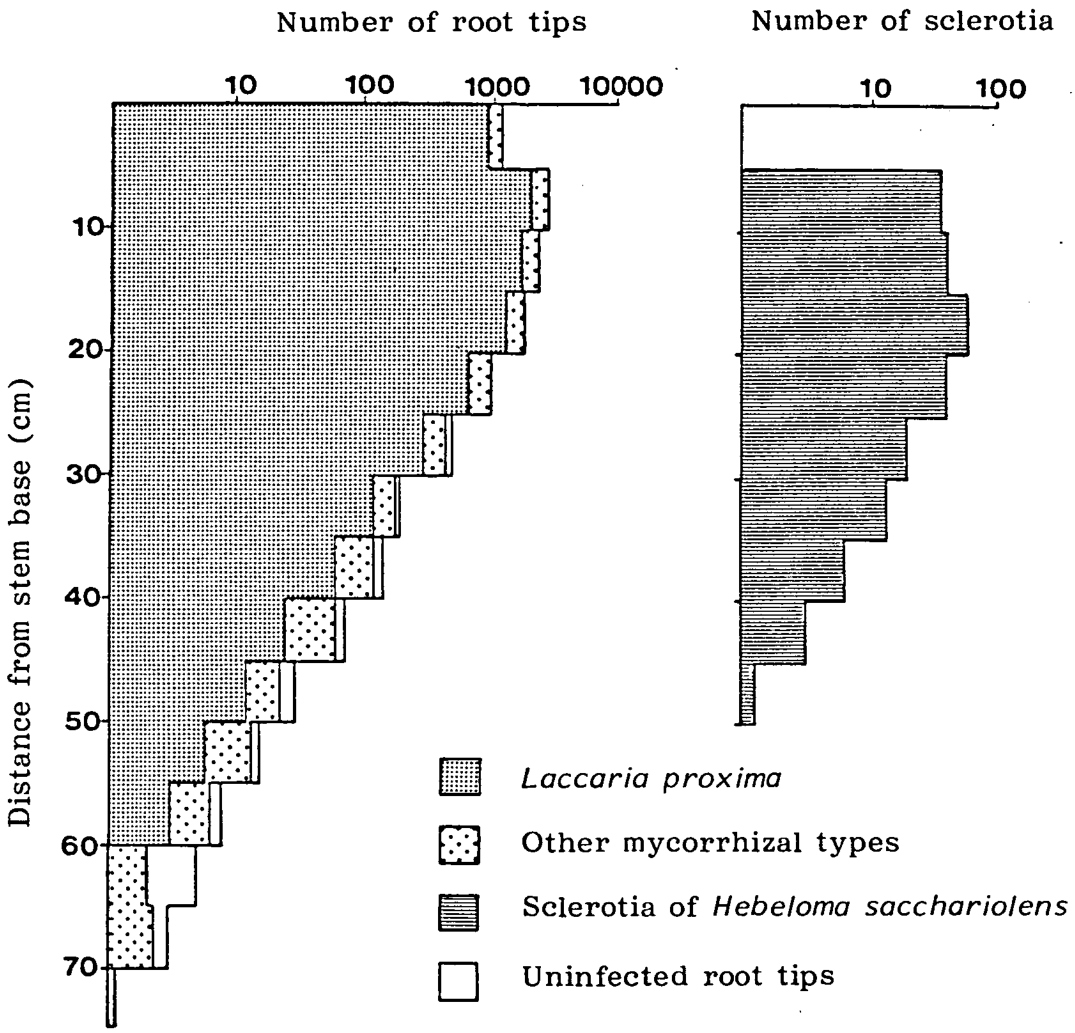

FIGURE 4.2.1: Distribution of mycorrhizas, and the sclerotium-like bodies of *Hebeloma sacchariolens*, down the root systems of birch seedlings propagated with *Laccaria proxima* and grown for 441 days in brown earth supplemented with inoculum of different mycorrhizal fungi.

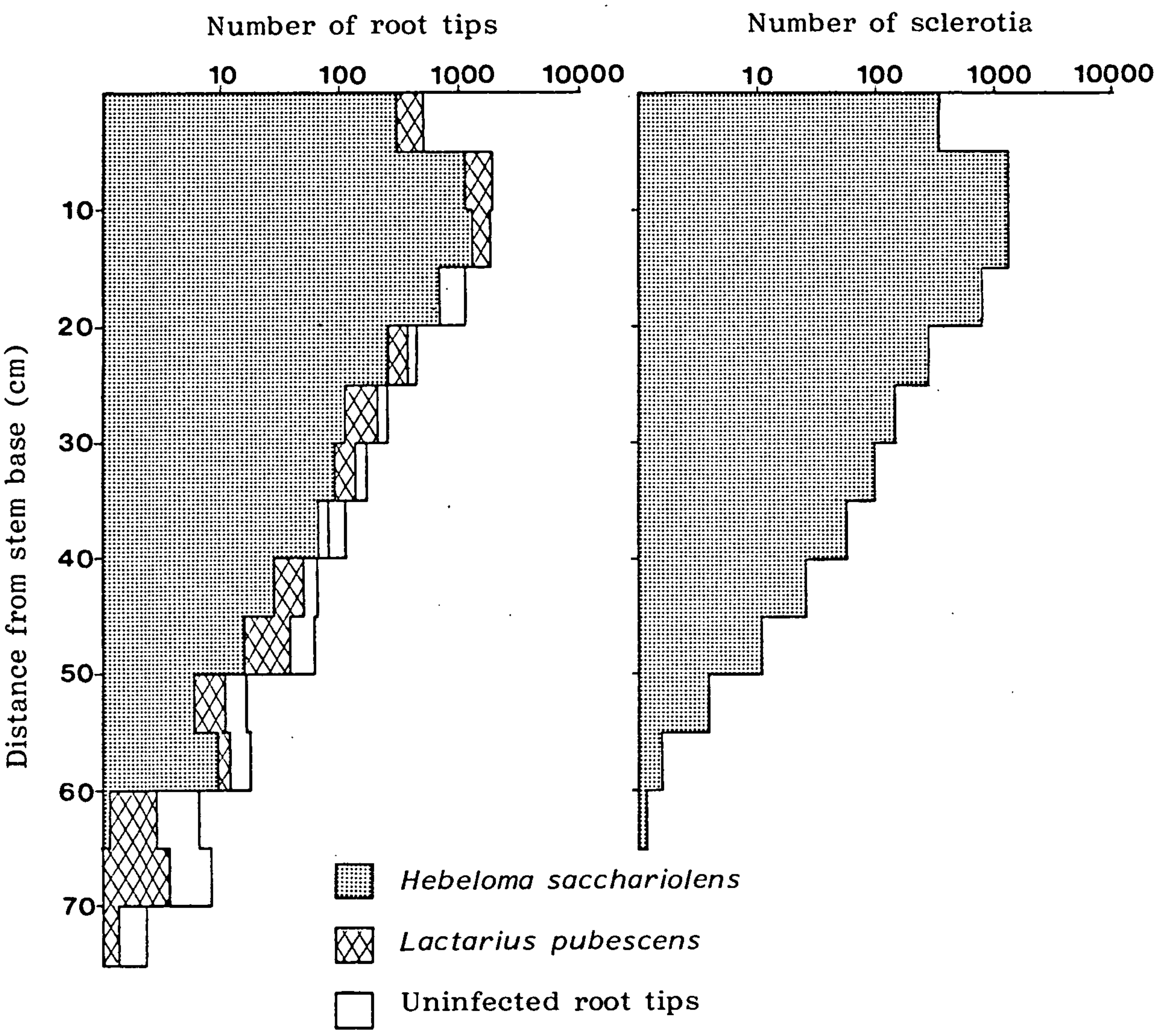

FIGURE 4.2.2: Distribution of mycorrhizas, and the sclerotium-like bodies of *Hebeloma sacchariolens*, down the root systems of birch seedlings propagated with *H. sacchariolens* and grown for 441 days in brown earth supplemented with inoculum of different mycorrhizal fungi.

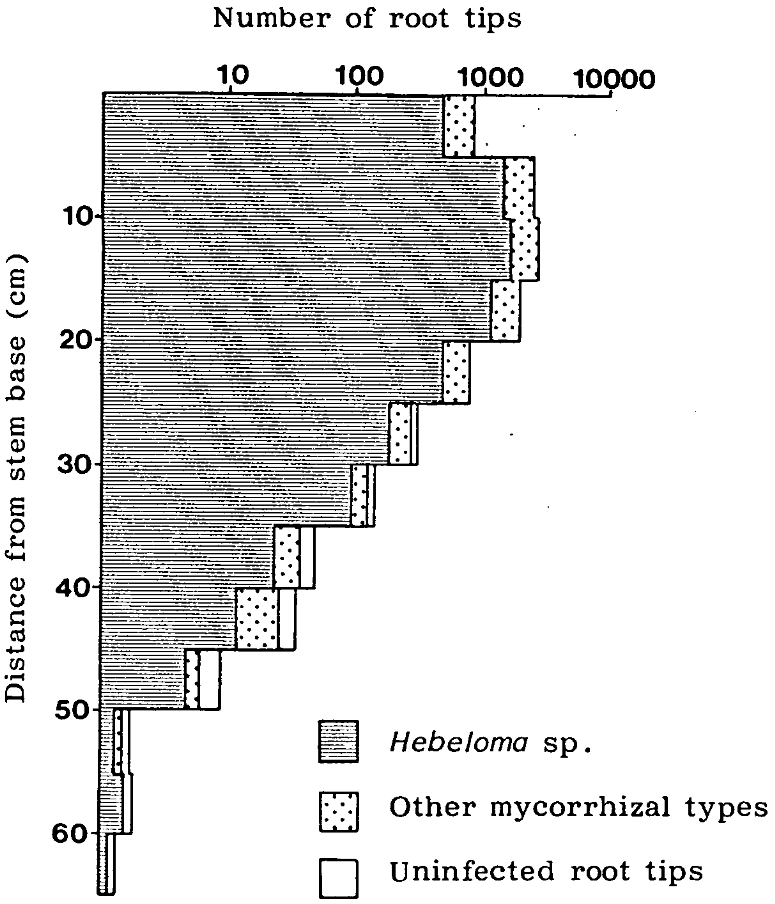

FIGURE 4.2.3: Distribution of mycorrhizas down the root systems of originally non-mycorrhizal birch seedlings grown for 441 days in brown earth supplemented with inoculum of different mycorrhizal fungi.

comments made above; in particular, it is clear that the inoculant fungi progressively colonised the outgrowing roots and repressed the naturally occurring mycorrhizal types at most distances from the seedling base. In addition, Figure 4.2.2 shows that the sclerotium-like bodies of *H. sacchariolens*, by which the mycorrhizas of this fungus can reliably be recognised, were distributed in almost exactly similar manner and proportions to the mycorrhizas themselves. It thus seems that the sclerotium-like bodies are formed consistently and early in the course of mycorrhizal development. A corollary of this is that the mycorrhizas on the control (uninoculated) seedlings, although mainly referable to *Hebeloma* sp., were not formed by *H. sacchariolens* because they were not associated with sclerotium-like bodies (except in one instance, Table 4.2.4). Thus, although Hebeloma-type mycorrhizas were predominant in both the control and the *H. sacchariolens* inoculation treatments, the species involved in these associations were different - one persisting from the original inoculum and the other developing from natural soil-borne or air-borne inoculum.

No mycorrhizas attributable to *A. muscaria* or *L. scabrum* were seen at any time in the experiment, even in soil to which inoculum of these fungi had been added. In contrast, *L. pubescens*, which was also used as a soil supplement, was seen to have colonised some seedling roots by 117 days after transplanting (Table 4.2.5). Yet its degree of development on the seedlings was independent of soil-supplementation treatment (Table 4.2.5), strongly suggesting that the Lactarius-type mycorrhizas on the seedlings had developed from inoculum sources other than the vermiculite-peat inoculum of *L. pubescens* added to one batch of soil. In contrast to the lack of clear differences between the soil-supplementation treatments on the incidence or degree of mycorrhizal

TABLE 4.2.5: Mean numbers of Lactarius-type mycorrhizas on seedlings initially inoculated with different mycorrhizal fungi and grown in soils supplemented with other mycorrhizal fungi; pooled data for seedling inoculation or soil supplementation treatments, respectively.

Days after transplanting	Soil inoculum addition			
	None	*Lactarius pubescens*	*Leccinum scabrum*	*Amanita muscaria*
117	0 *(0.000)*	36 *(0.493)*	155 *(0.349)*	11 *(0.296)*
190	7 *(0.325)*	190 *(0.759)*	74 *(0.513)*	0 *(0.000)*
441	641 *(1.197)*	1572 *(1.051)*	1092 *(1.187)*	2201 *(2.313)*

Days after transplanting	Seedling inoculant		
	Hebeloma sacchariolens	*Laccaria proxima*	None
117	34 *(0.429)*	1 *(0.163)*	117 *(0.364)*
190	200 *(0.709)*	2 *(0.124)*	42 *(0.507)*
441	1965^{a} *(1.571)*	0^{ab} *(0.000)*	2147^{b} *(2.008)*

* Figures in each row followed by the same letter differ from one another at $p = 0.05$; analysis by Student-Newman-Keuls test.

development by *L. pubescens*, pre-inoculation treatments had significant and interesting effects (Table 4.2.5). In particular, seedlings pre-inoculated with *L. proxima* before transplanting into soil developed few or no Lactarius-type mycorrhizas, whereas such mycorrhizas did develop on control seedlings (originally non-mycorrhizal) or on seedlings inoculated with *H. sacchariolens*. The reason for this is unknown.

Of particular interest, large numbers of mycorrhizas attributable to *L. pubescens* developed only after the seedlings had gone through the imposed dormancy (Table 4.2.5), though the results do not necessarily suggest that there was a causal relationship in this respect. At 441 days, Lactarius-type mycorrhizas were seen on 6 of 11 seedlings propagated with *H. sacchariolens* and 9 out of 12 originally non-mycorrhizal (control) seedlings; these mycorrhizas accounted for 22.1% of the root tips on the control seedlings and 29.8% of those on the seedlings propagated with *H. sacchariolens*. Indeed, comparison of Tables 4.2.4 and 4.2.5 shows that mycorrhizas of *L. pubescens* accounted for all of the naturally occurring types (termed 'others' in Table 4.2.4) in the *H. sacchariolens* propagation treatment. In detailed microscopical observations, the mycorrhizas of *L. pubescens* were seen to occupy the sites of some of the former mycorrhizas of *H. sacchariolens*, of which only the sclerotium-like bodies remained (Plate 4.1). A further point of interest is that in the final sample, 8 out of the 10 remaining seedlings that had initially been inoculated with *L. proxima* bore the mycorrhizas of *Cenococcum geophilum*; these seedlings bore, on average, 421 ± 214.4 (s.e.) mycorrhizal tips formed by this fungus. This was the only occasion, in this experiment in which this mycorrhizal type was observed.

PLATE 4.1: Mycorrhizas of *Lactarius pubescens* (arrowed) formed on the tip of a mycorrhiza formed by *Hebeloma sacchariolens*, identifiable by the presence of white, globose sclerotium-like bodies.

Fruitbodies of *T. terrestris* developed in mid-September 1981 in 6 of 36 pots that had been planted with initially non-mycorrhizal seedlings; no fruitbodies of this fungus were observed in pots containing inoculated seedlings. *H. sacchariolens* produced fruitbodies late in October in only 2 of 24 pots that contained seedlings initially inoculated with this fungus; starting late in November, fruitbodies of *L. proxima* were observed in 10 of 22 pots that contained seedlings inoculated with this fungus. The appearance of fruitbodies thus helped to confirm the identification of mycorrhizal types and to establish that the inoculant fungi were still present.

In summary of this experiment, it was shown that two mycorrhizal fungi, *H. sacchariolens* and *L. proxima*, can persist once established on seedling roots and can colonise newly formed roots on the seedlings for up to 441 days after transplanting to a brown earth soil. Furthermore, these inoculant mycorrhizal fungi remained dominant throughout the experiment, and colonisation of the seedling roots from naturally occurring sources of inoculum of mycorrhizal fungi was correspondingly reduced. Addition of inoculum of *L. scabrum* or *A. muscaria* to the soil did not result in formation of mycorrhizas by these fungi, so there was no evidence of a 'priming' effect of 'early-stage' mycorrhizal fungi on the establishment of 'late-stage' ones. Although *L. pubescens* formed mycorrhizas on the seedlings, their occurrence bore no relation to the addition to soil of inoculum of *L. pubescens*; the fungus thus developed mycorrhizas from naturally occurring sources of inoculum. The numbers of this mycorrhizal type increased greatly in the second growing season but these mycorrhizas were not observed on seedlings that had been inoculated initially with *L. proxima*.

4.2.2 *The Effect of Prolonged Incubation of Seedlings in Soil Cores on the Formation of Mycorrhizas by Some 'Late-Stage' Fungi*

Deacon *et al.* (1983) observed little or no infection of seedlings by 'late-stage' mycorrhizal fungi when seedlings were grown in soil cores taken from beneath fruitbodies of these fungi. However, Deacon *et al.* (1983) incubated the cores for only 17 weeks at most. There was some indication from their experiments that mycorrhizas of *L. pubescens* started to develop towards the end of this period. The present experiment was therefore done to see if incubation of the cores for longer periods than 17 weeks results in the development of such 'late-stage' mycorrhizal fungi.

Twenty soil cores (8 x 2 cm diameter) were collected on 17 January 1982 on the Bush Estate, from each of the positions where the fruitbodies of *Lactarius pubescens*, *Leccinum* sp.[1] and *Cortinarius* sp. had been observed in the previous autumn. A further twenty cores, to act as controls, were collected at least 5 m away from any trees. Four of the cores in each category were randomly selected and examined for mycorrhizas. The remaining ones were trimmed so that they would fit into clean polystyrene containers (8 x 2 cm internal diameter) and each core was then planted, on 18 January, with an aseptically germinated birch seedling. The cores were randomised and stood upright in trays of moist sand, incubated in a growth room (Section 2.4.1). Six seedlings from each category were randomly selected and sampled on 23 April 1982, after 95 days' growth. The remaining cores were then removed from their containers and transferred to 100 mm diameter plant pots containing 'non-mycorrhizal' Bush soil and placed in a heated

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[1] These fruitbodies correspond to material that Dr. R. Watling, Royal Botanical Garden, Edinburgh, intends to describe as *Leccinum rigidipes* (R. Watling, pers. comm.).

glasshouse (max. 28°C, min. 14°C). The plants were harvested after a further 184 days' growth (24 November 1982) and the mycorrhizal types on the root systems were assessed qualitatively.

There was no significant difference in oven-dry weight of the seedling shoots at either of the sampling dates (Table 4.2.6); but at both sampling times the seedlings grown in the soil cores taken from beneath the fruitbodies of *Leccinum* sp. were significantly taller than the other seedlings. There was no significant difference between the treatments in the total number of root tips at the time of the first sampling (Table 4.2.7) but there were some interesting and marked differences in the mycorrhizal types observed in the different treatments. The majority of mycorrhizal types observed on the seedlings after 95 days were formed by *Thelephora terrestris*, *Hebeloma* sp. and *Inocybe* sp., that is by fungi that can be considered 'early-stage' by the criterion of Deacon *et al.* (1983); the mycorrhizal types grouped together as 'others' in Table 4.2.7 include Laccaria-type mycorrhizas and two unidentified types. Seedlings grown in soil cores taken from beneath the fruitbodies of *Cortinarius* sp. bore mainly 'type 5' mycorrhizas (Table 4.2.7); these cores had been collected around a tree (termed N2) used in another experiment in this thesis (Section 5.2.1) and in which, again, type 5 mycorrhizas were common. Type 5 mycorrhizas might be attributable to *Cortinarius* sp. With this possible exception, Table 4.2.7 shows that mycorrhizas of the 'fruiting fungi' did not develop on the seedling roots by 95 days; rather, the seedlings grown in soil cores from beneath fruitbodies of *L. pubescens* and *Leccinum* sp. developed mycorrhizas predominantly attributable to *T. terrestris*, whereas seedlings in control cores, taken away from trees, developed other mycorrhizal types.

TABLE 4.2.6: Mean shoot heights and oven-dry weights of birch seedlings grown in soil cores taken away from trees or beneath the fruitbodies of mycorrhizal fungi; data transformed to log(x+1) for analysis (in parentheses).*

			Soil cores		
Seedling age (days)		Control	*Cortinarius* sp.	*Lactarius pubescens*	*Leccinum* sp.
95	Height (mm)	30^{a} *(1.449)*	34^{b} *(1.486)*	39^{ab} *(1.587)*	57^{ab} *(1.763)*
	Dry weight (mg)	8.2 *(0.860)*	10.7 *(0.851)*	15.5 *(1.102)*	16.5 *(1.186)*
279	Height (mm)	130^{a} *(2.093)*	119^{b} *(2.066)*	123^{c} *(2.073)*	131^{abc} *(2.118)*
	Dry weight (mg)	577.7 *(2.715)*	528.1 *(2.712)*	534.4 *(2.717)*	591.7 *(2.769)*

* Figures in each row followed by the same letter differ from one another at p = 0.05.

TABLE 4.2.7: Mean numbers (± s.e.) of root tips of different mycorrhizal types on birch seedlings grown for 95 days in soil cores taken from beneath the fruitbodies of mycorrhizal fungi.

		Soil cores		
Mycorrhizal type on seedlings	Control (n=6)	*Cortinarius* sp. (n=5)	*Lactarius pubescens* (n=4)	*Leccinum* sp. (n=6)
Thelephora	9 ± 5.5	trace	45 ± 16.6	100 ± 21.1
Hebeloma	10 ± 4.7	2 ± 1.4	8 ± 8.0	2 ± 1.4
Inocybe	0	0	17 ± 17.5	0
Type 5	0	53 ± 17.5	4 ± 3.4	0
Others	47 ± 19.6	20 ± 16.3	2 ± 2.5	0
None (uninfected)	5 ± 2.3	2 ± 1.0	20 ± 9.5	3 ± 1.7
Total	71 ± 18.7	77 ± 27.7	96 ± 32.8	105 ± 21.8

The mycorrhizas that were most frequently dominant on the seedlings at the second sampling date were again of typical 'early-stage' fungi (Deacon *et al.*, 1983), namely *Hebeloma* sp., *T. terrestris*, *Inocybe* sp. and *Laccaria* sp. (Table 4.2.8). Of these, Hebeloma-type mycorrhizas were found most frequently in 'control', 'Cortinarius' and 'Lactarius' cores but not in those from beneath *Leccinum* sp., which had predominantly Inocybe-type mycorrhizas. Laccaria-type mycorrhizas were common in the 'control' and 'Lactarius' cores but not in the 'Cortinarius' and 'Leccinum' cores, whereas *T. terrestris* was more uniformly distributed between treatments (Table 4.2.8). For reasons that are unclear, these patterns of distribution between treatments do not closely parallel those seen at the 95 day sampling. Mycorrhizas of *Leccinum* sp. were observed on only one seedling, and this had been grown in a soil core taken from beneath a fruitbody of *L. pubescens*. Similarly, although the mycorrhizas of *L. pubescens* were observed on 9 seedlings, and were dominant on 5 of them, only one of these seedlings was grown in a soil core taken from beneath a fruitbody of this fungus; however, the majority of seedlings infected by *L. pubescens* were grown in the 'Cortinarius' cores which were collected from around a tree (N2) known to bear the mycorrhizas of *L. pubescens* (Section 5.2.1). Of especial interest, the mycorrhizas of type 5, which were common at 95 days on seedlings grown in cores from beneath fruitbodies of *Cortinarius* sp., were not seen at the second sampling. A further point of interest is that the mycorrhizas of *H. sacchariolens*, identified by their white sclerotium-like bodies, were present and dominant, on three seedlings from the 'Lactarius' treatment. Unfortunately, the results from the dissection cores are not available so it is not possible to relate the occurrence of mycorrhizas on the

TABLE 4.2.8: Numbers of seedlings, grown for 279 days in soil cores taken from beneath the fruitbodies of different mycorrhizal fungi, on which different mycorrhizal types were present, dominant or had produced fruitbodies.

Mycorrhizal type on seedlings	Control cores (max. 9)			*Leccinum* cores (max. 10)		
	present	dominant	fruiting	present	dominant	fruiting
Hebeloma	7	5	2	1	1	0
Laccaria	8	2	3	0	0	0
Inocybe	2	0	0	7	6	0
Lactarius	2	1	0	0	0	0
Thelephora	2	1	0	6	3	5
Cenococcum	1	0	0	1	0	0
	Lactarius pubescens cores (max. 9)			*Cortinarius* cores (max. 10)		
	present	dominant	fruiting	present	dominant	fruiting
Hebeloma	6	2	0	10	7	1
Laccaria	5	1	2	1	0	0
Inocybe	2	1	0	0	0	0
Lactarius	1	1	0	6	2	0
Thelephora	5	1	1	5	1	3
Hebeloma sacchariolens	3	3	0			
Leccinum	1	0	0			
Paxillus	1	1	0			

seedlings to the original occurrence of mycorrhizas in soil cores. However, Deacon *et al.* (1983), working at the same site, always found the mycorrhizas of *L. pubescens* and *Leccinum* spp. in soil cores beneath their respective fruitbodies, so it may be assumed that this was the case in the present experiment.

A number of fruitbodies was observed in the pots late in October, as indicated in Table 4.2.8, and where they occurred they matched the occurrence of mycorrhizas on the root systems.

In summary of this experiment, it is clear that prolonged incubation resulted in the colonisation of some seedlings by *L. pubescens*, though the frequency of occurrence of mycorrhizas of this fungus was low and was not necessarily related to the origin of the soil cores. In this respect, these results parallel those obtained in Section 4.2.1. The other 'late-stage' mycorrhizal fungi did not develop to a significant degree even on prolonged incubation of seedlings, though it is unclear whether type 5 mycorrhizas, seen on seedlings in cores from beneath fruitbodies of *Cortinarius* sp. were attributable to this fungus. Despite the failure of this experiment in terms of its primary objective, it nevertheless showed interesting differences between the treatments in the establishment of several 'early-stage' mycorrhizal fungi.

4.3 Interactions Between Mycorrhizal Fungi from Soil-borne or Seedling-borne Inoculum

In a previous experiment it was demonstrated that two mycorrhizal fungi, *Laccaria proxima* and *Hebeloma sacchariolens*, once established on seedling roots, prevented colonisation of the roots by other mycorrhizal fungi present at relatively high inoculum levels in soil (Section 4.2.1). However, all of the ineffective soil inocula in that experiment were of 'late-stage' mycorrhizal fungi, so it remained possible that 'early-stage' fungi might colonise the roots from soil in similar conditions even though the seedlings already bore mycorrhizas. The present experiments were designed to test this possibility and thus also to test the abilities of pre-established mycorrhizal fungi to persist on roots of seedlings transplanted into inoculum-supplemented soils. The three fungi chosen for the experiments were:

1. *Paxillus involutus*, a fungus frequently found on poor sites such as coal spoil heaps and potentially useful for inoculation programmes (Mason *et al.*, 1983a);
2. *H. sacchariolens*, a fungus observed on birch growing in brown earth on the Bush Estate and known to be effective in reducing colonisation of roots by other mycorrhizal fungi present as natural inoculum in soil (Section 4.2.1);
3. *Thelephora terrestris*, a fungus commonly found in tree nurseries (Marx, 1980) and so likely to be present on many seedlings transplanted to the field in commercial practice.

These three fungi were especially useful because they form distinctive and easily recognisable mycorrhizal types.

4.3.1 *Competitive Interactions Between Three Mycorrhizal Fungi in Brown Earth*

Pre-germinated birch seedlings were aseptically transferred on 16 December 1981 to propagators (Section 2.3.2) containing inoculum of *P. involutus* (isolate 16), *T. terrestris* (isolate 1), *H. sacchariolens* (isolate 4) or autoclaved inoculum (a mixture of the three fungi). The seedlings were then incubated for 89 days, when they were ready for transplanting, having established on their roots mycorrhizas attributable to the 'primary inoculants'. On 11 March 1982, 'non-mycorrhizal' Bush soil was collected and mixed with vermiculite-peat inoculum of the same three fungi or with autoclaved inoculum (3 : 1, v/v) and these supplemented batches of soil were added to 75 mm diameter plant pots. The seedlings from pure culture were transferred to these pots on 13 March 1982, all pre-inoculation treatments being combined factorially with all soil supplementation treatments, and the pots were incubated in nine randomised blocks in a heated glasshouse (max. 28°C, min. 13°C). Four blocks were randomly selected and harvested on 23 May 1982 after 71 days' growth, but because some of the seedlings had died extra replicates were taken, if necessary, from 'spare' blocks. Three more blocks were sampled on 20 August 1982, after 170 days. At each sampling the shoot heights and dry weights of the seedlings were recorded, as were the numbers of mycorrhizal root tips in successive 3 cm long pieces of root, working outwards from the stem base (Section 2.5.2c).

At the time of transplanting the seedlings inoculated with *P. involutus* were tallest, but otherwise there was little difference between the seedlings in shoot dry weight or the total number of root tips (Table 4.3.1). All the inoculated seedlings bore mycorrhizas of the appropriate type whereas the control seedlings were non-mycorrhizal.

TABLE 4.3.1: Mean (± s.e.) shoot height and oven-dry weight and numbers of root tips on seedlings incubated for 89 days with inoculum of three mycorrhizal fungi.

	Seedling inoculation			
	Paxillus involutus (n=3)	*Thelephora terrestris* (n=3)	*Hebeloma sacchariolens* (n=4)	None (n=3)
Height (mm)	45 ± 6.2	18 ± 2.7	26 ± 2.6	26 ± 7.6
Dry weight (mg)	3.0 ± 1.08	7.1± 7.10*	1.7 ± 0.25	6.2 ± 2.58
Mycorrhizal root tips	19 ± 2.6	33 ± 10.1	17 ± 0.5	0
Uninfected root tips	20 ± 11.7	14 ± 4.2	22 ± 4.7	56 ± 29.4
Total root tips	40 ± 12.7	47 ± 13.0	40 ± 5.1	56 ± 29.4

* only one replicate was weighed.

After 71 days in soil the seedlings initially inoculated with *H. sacchariolens* had a significantly lower dry weight, when averaged across the soil supplementation ('secondary inoculant') treatments, than had the others and were also the smallest (Table 4.3.2). Seedlings originally inoculated with *P. involutus* remained tallest, but there was no effect of different soil inocula, averaged across the pre-inoculation treatments, on seedling height (Table 4.3.2). Seedlings grown in soil supplemented with *H. sacchariolens* were significantly heavier than seedlings grown in the control soil.

After 170 days in soil there were no differences in shoot dry weight between any of the treatments, and although seedlings pre-inoculated with *P. involutus* were tallest, yet the difference was not, in general, statistically significant (Table 4.3.3).

The fungi showed notable differences both in ability to persist as primary inoculants and in ability to colonise seedling roots as secondary inoculants (Table 4.3.4). *P. involutus* had not colonised seedlings from soil-borne inoculum at either sampling time, and after 71 days in soil it had not even maintained itself on all of the seedlings on which it was initially present (Table 4.3.4). At this stage, *P. involutus* was present on a greater number of seedlings, initially pre-inoculated with this fungus, that had been transplanted into control soil and soil supplemented with *P. involutus* than it was on seedlings transplanted into soil containing inoculum of other fungi. After 170 days very few seedlings inoculated with *P. involutus* bore any mycorrhizas of this fungus. By contrast, all of the seedlings inoculated with *H. sacchariolens* or *T. terrestris* or transplanted into soil containing inoculum of these fungi bore their mycorrhizas at both sampling times (Table 4.3.4).

TABLE 4.3.2: Mean height and oven-dry weight of seedlings initially propagated with three mycorrhizal fungi and then grown for 71 days in soil supplemented with inoculum of mycorrhizal fungi*; data (a) pooled for soil supplementation treatments or (b) pooled for seedling inoculation treatments, and transformed to log(x+1) for analysis (in parentheses).

	Paxillus involutus	*Thelephora terrestris*	*Hebeloma sacchariolens*	None	L.S.D. (p = 0.05)
(a) Seedling inoculation					
Height (mm)	55 *(1.739)*	34 *(1.526)*	30 *(1.481)*	51 *(1.708)*	*(0.071)*
Dry weight (mg)	59.2 *(1.765)*	61.6 *(1.698)*	30.9 *(1.430)*	97.9 *(1.985)*	*(0.156)*
(b) Soil inoculum addition					
Height (mm)	44 *(1.638)*	45 *(1.625)*	40 *(1.588)*	42 *(1.602)*	*(0.071)*
Dry weight (mg)	63.2 *(1.760)*	59.1 *(1.653)*	70.8 *(1.816)*	56.4 *(1.648)*	*(0.156)*

* see text for explanation.

TABLE 4.3.3: Mean shoot height and oven-dry weight of seedlings initially propagated with three mycorrhizal fungi and then grown for 170 days in soil supplemented with mycorrhizal fungi*; data (a) pooled for soil supplementation treatments or (b) pooled for seedling inoculation treatments, and transformed to log(x+1) for analysis (in parentheses).

	Paxillus involutus	*Thelephora terrestris*	*Hebeloma sacchariolens*	None	L.S.D. ($p = 0.05$)
(a) <u>Seedling inoculation</u>					
Height (mm)	72 *(1.845)*	53 *(1.707)*	53 *(1.757)*	58 *(1.762)*	*(0.094)*
Dry weight (mg)	213 *(2.285)*	172 *(2.229)*	183 *(2.212)*	196 *(2.272)*	*(0.183)*
(b) <u>Soil inoculum addition</u>					
Height (mm)	54 *(1.698)*	55 *(1.755)*	66 *(1.826)*	61 *(1.792)*	*(0.094)*
Dry weight (mg)	182 *(2.252)*	170 *(2.241)*	231 *(2.251)*	183 *(2.256)*	*(0.183)*

* for explanation see text.

TABLE 4.3.4: Numbers of seedlings colonised by primary inoculant* type (x/-) and secondary inoculant* type (-/x) mycorrhizas after being incubated in soil for 71 days (max. 4 replicate seedlings) or 170 days (max. 3 replicate seedlings).

Days after transplanting	Soil (secondary) inoculant	Seedling (primary) inoculant			
		Paxillus involutus	*Thelephora terrestris*	*Hebeloma sacchariolens*	*None*
71	*P. involutus*	3 / -	4 / 0	4 / 0	- / 0
	T. terrestris	1 / 4	4 / -	4 / 4	- / 4
	H. sacchariolens	2 / 4	4 / 4	4 / -	- / 4
	None	4 / -	4 / -	4 / -	- / -
170	*P. involutus*	1 / -	3 / 0	3 / 0	- / 0
	T. terrestris	1 / 3	3 / -	3 / 3	- / 3
	H. sacchariolens	1 / 3	3 / 3	3 / -	- / 2**
	None	0 / -	3 / -	3 / -	- / -

* for explanation see text.

** maximum of two replicates only.

Further details of the degree of mycorrhizal development in different treatments are given in Tables 4.3.5 and 4.3.6. It is seen that, even when present, the mycorrhizas of *P. involutus* were few in number and the majority of root tips on the seedlings, with *P. involutus* as primary inoculant, were formed by secondary inoculants or naturally occurring fungi (Tables 4.3.5 and 4.3.6). Apart from the presence of some Paxillus-type mycorrhizas, therefore, mycorrhizal development on these seedlings closely paralleled that on the initially non-mycorrhizal (control) seedlings. Mycorrhizal types, referred to as 'others' (Tables 4.3.5 and 4.3.6), observed on the control seedlings were formed predominantly by *Hebeloma* sp. or *Laccaria* sp. In contrast, mycorrhizas of *H. sacchariolens* (the identity of which was confirmed by the presence of sclerotium-like bodies) were dominant on all of the seedlings exposed to either primary or secondary inoculum of this fungus - even if the seedlings had previously established mycorrhizas with other primary inoculants (Tables 4.3.5 and 4.3.6). In the absence of *H. sacchariolens*, *T. terrestris* became dominant as both primary and secondary inoculant - for example, on seedlings initially inoculated with *P. involutus*.

Statistical analyses of some of the results, based on the percentage of root tips on individual seedlings occupied by primary or secondary inoculant fungi, are presented in Table 4.3.7. It is seen that, when present as a primary inoculant, *H. sacchariolens* continued to occupy a similar proportion of the root system irrespective of the addition of secondary inoculants to the soil, whereas *T. terrestris* was suppressed by *H. sacchariolens* growing from secondary inoculum. Similarly, secondary inoculum of *H. sacchariolens* was able to establish mycorrhizas irrespective of the presence of primary inoculant fungi, whereas

TABLE 4.3.5: Mean numbers (± s.e.) of primary and secondary inoculant* type mycorrhizas and other mycorrhizal types on seedlings grown in soil for 71 days.

Soil (secondary) inoculant	Mycorrhizal types present***	Seedling (primary) inoculant			
		Paxillus involutus	*Thelephora terrestris*	*Hebeloma sacchariolens*	None
Paxillus involutus	1°	47 ± 44.1	549 ± 110.5	153 ± 24.8	-
	2°	-	0	0	0
	others	0	0	2 ± 2.2	291 ± 185.8
	none	895 ± 162.2	389 ± 113.2	145 ± 34.4	928 ± 137.2
Thelephora terrestris	1°	5 ± 5.5	321 ± 97.8	137 ± 44.9	-
	2°	356 ± 63.2	-	33 ± 19.6	668 ± 136.7
	others	0	0	0	0
	none	384 ± 85.8	332 ± 61.0	83 ± 29.4	515 ± 122.8
Hebeloma sacchariolens	1°	23 ± 21.1	25 ± 10.4	223 ± 30.3	-
	2°	403 ± 98.7	202 ± 61.0	-	422 ± 68.8
	others	0	0	tr**	0
	none	129 ± 24.4	217 ± 69.4	162 ± 10.5	151 ± 24.0
None	1°	232 ± 80.7	430 ± 300.3	158 ± 84.0	-
	2°	-	-	-	-
	others	0	0	0	348 ± 157.9
	none	433 ± 109.8	170 ± 60.1	136 ± 68.0	1047 ± 81.2

* for explanation see text; ** tr, <1 mycorrhizal tip per seedling.

*** 1°, primary inoculant type mycorrhizas; 2°, secondary inoculant type mycorrhizas; others, other mycorrhizal types; none, uninfected root tips.

TABLE 4.3.6: Mean numbers (± s.e.) of primary and secondary inoculant type mycorrhizas* and other mycorrhizal types on seedlings grown in soil for 170 days.

Soil (secondary) inoculant	Mycorrhizal types present**	Seedling (primary) inoculant			
		Paxillus involutus	*Thelephora terrestris*	*Hebeloma sacchariolens*	None
Paxillus involutus	1°	21 ± 20.7	718 ± 140.0	1252 ± 148.1	-
	2°	-	0	0	0
	others	1067 ± 234.8	85 ± 15.5	0	1057 ± 143.5
	none	69 ± 15.0	179 ± 128.8	73 ± 7.0	57 ± 12.2
Thelephora terrestris	1°	118 ± 118.3	1015 ± 227.8	560 ± 169.2	-
	2°	938 ± 210.8	-	387 ± 125.8	1296 ± 141.2
	others	315 ± 164.9	185 ± 113.8	4 ± 4.0	168 ± 88.1
	none	337 ± 181.8	39 ± 18.0	66 ± 15.3	49 ± 6.8
Hebeloma sacchariolens	1°	20 ± 19.7	841 ± 235.4	695 ± 242.9	-
	2°	553 ± 230.2	1174 ± 339.5	-	1426 ± 442.1
	others	111 ± 111.0	52 ± 17.8	0	158 ± 158.5
	none	147 ± 34.6	131 ± 22.9	1236 ± 195.2	66 ± 23.5
None	1°	0	1092 ± 97.6	1236 ± 195.2	-
	2°	-	-	-	-
	others	1140 ± 383.0	142 ± 44.0	0	-
	none	52 ± 12.2	74 ± 12.7	102 ± 18.8	-

* for explanation see text.

** 1°, primary inoculant type mycorrhizas; 2°, secondary inoculant type mycorrhizas; others, other mycorrhizal types; none, uninfected root tips.

TABLE 4.3.7: Mean percentage of roots occupied by (a) primary or (b) secondary inoculant type mycorrhizas on seedlings initially propagated with mycorrhizal fungi and grown for 170 days in soil supplemented with mycorrhizal fungi; data transformed to angles for analysis (in parentheses).

Seedling (primary) inoculant	Soil (secondary) inoculant				
	Paxillus involutus	*Thelephora terrestris*	*Hebeloma sacchariolens*	None	L.S.D. (p = 0.05)
(a) Primary inoculant mycorrhizas on seedlings					
Paxillus involutus	1.9 *(3.5)*	11.8 *(9.1)*	1.6 *(3.1)*	0.0 *(0.0)*	*(22.3)*
Thelephora terrestris	75.8 *(61.1)*	80.5 *(65.3)*	38.4 *(38.1)*	80.9 *(64.3)*	*(16.8)*
Hebeloma sacchariolens	94.5 *(76.4)*	53.8 *(47.2)*	85.7 *(68.4)*	92.1 *(73.8)*	*(14.4)*

Soil (secondary) inoculant	Seedling (primary) inoculant				
	Paxillus involutus	*Thelephora terrestris*	*Hebeloma sacchariolens*	None	L.S.D. (p = 0.05)
(b) Secondary inoculant mycorrhizas on seedlings					
Paxillus involutus	1.9 *(4.6)*	0.0 *(0.0)*	0.0 *(0.0)*	0.0 *(0.0)*	*(7.6)*
Thelephora terrestris	65.3 *(54.1)*	80.5 *(65.3)*	39.3 *(38.4)*	86.9 *(69.4)*	*(20.1)*
Hebeloma sacchariolens	79.5 *(64.0)*	52.5 *(46.5)*	68.4 *(68.4)*	- -	*(16.7)*

T. terrestris colonised poorly on root systems first colonised by *H. sacchariolens*. Interestingly, in no instance was the percentage infection by a fungus increased by secondary inoculum if the same fungus was already present as a primary inoculant.

Although the proportion of the root system occupied by a primary inoculant was sometimes reduced by the presence of another fungus, yet the primary inoculant could still spread out on the roots from the original sites of infection (Figures 4.3.1 and 4.3.2). For example, mycorrhizas of *P. involutus* were seen at a gross distance of 15 cm away from the stem bases after 170 days (Figure 4.3.1), and yet the cylinder of root material initially placed in each pot was only 6 cm long. Unfortunately, the significance of such observations is unclear, because the pots themselves were only 7 cm deep and 7.5 cm diameter and the root systems were therefore restricted. A final point of interest is that the direct replacement of one mycorrhizal type by another, such as described by Marks and Foster (1967), was not observed in this experiment, but two types were occasionally observed growing in close proximity (Plate 4.2).

In summary, this experiment demonstrates that mycorrhizas formed by an isolate of *P. involutus* on seedling roots did not spread with the growing root system after the seedlings were transplanted into soil; the new roots were colonised by other mycorrhizal fungi. Moreover, *P. involutus* failed to colonise seedlings from inoculum added to soil. By contrast, *H. sacchariolens* and *T. terrestris*, as either primary or secondary inoculants, colonised seedling roots extensively. Indeed, *H. sacchariolens* was always the dominant mycorrhizal fungus on the seedlings, when this fungus was introduced as seedling-borne or soil-borne inoculum, regardless of other seedling or soil inoculants. However,

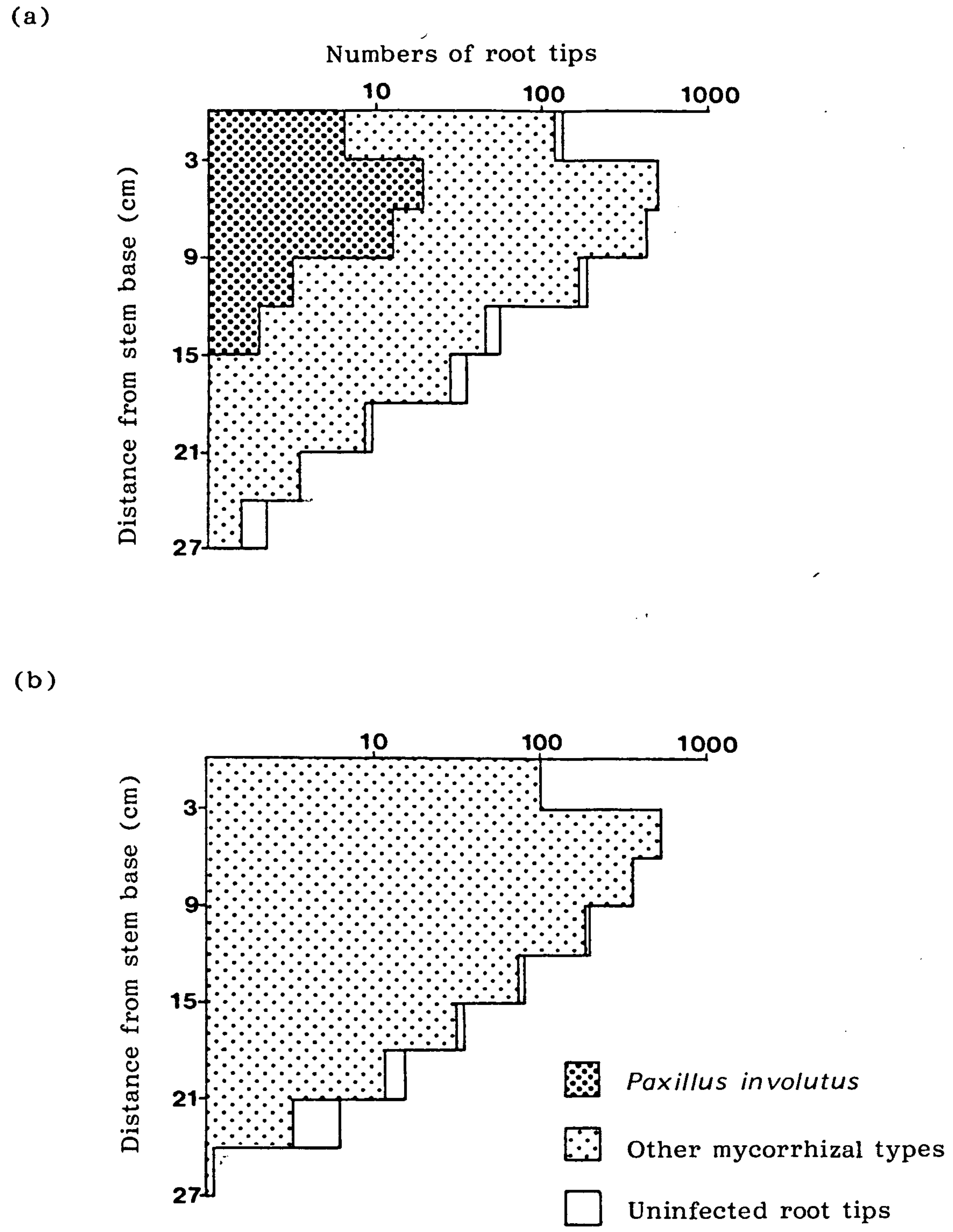

FIGURE 4.3.1: Distribution of mycorrhizas at different distances from the stem base of seedlings (a) initially inoculated with *Paxillus involutus* and (b) initially non-mycorrhizal when incubated for 170 days in soil containing the inocula of other mycorrhizal fungi.

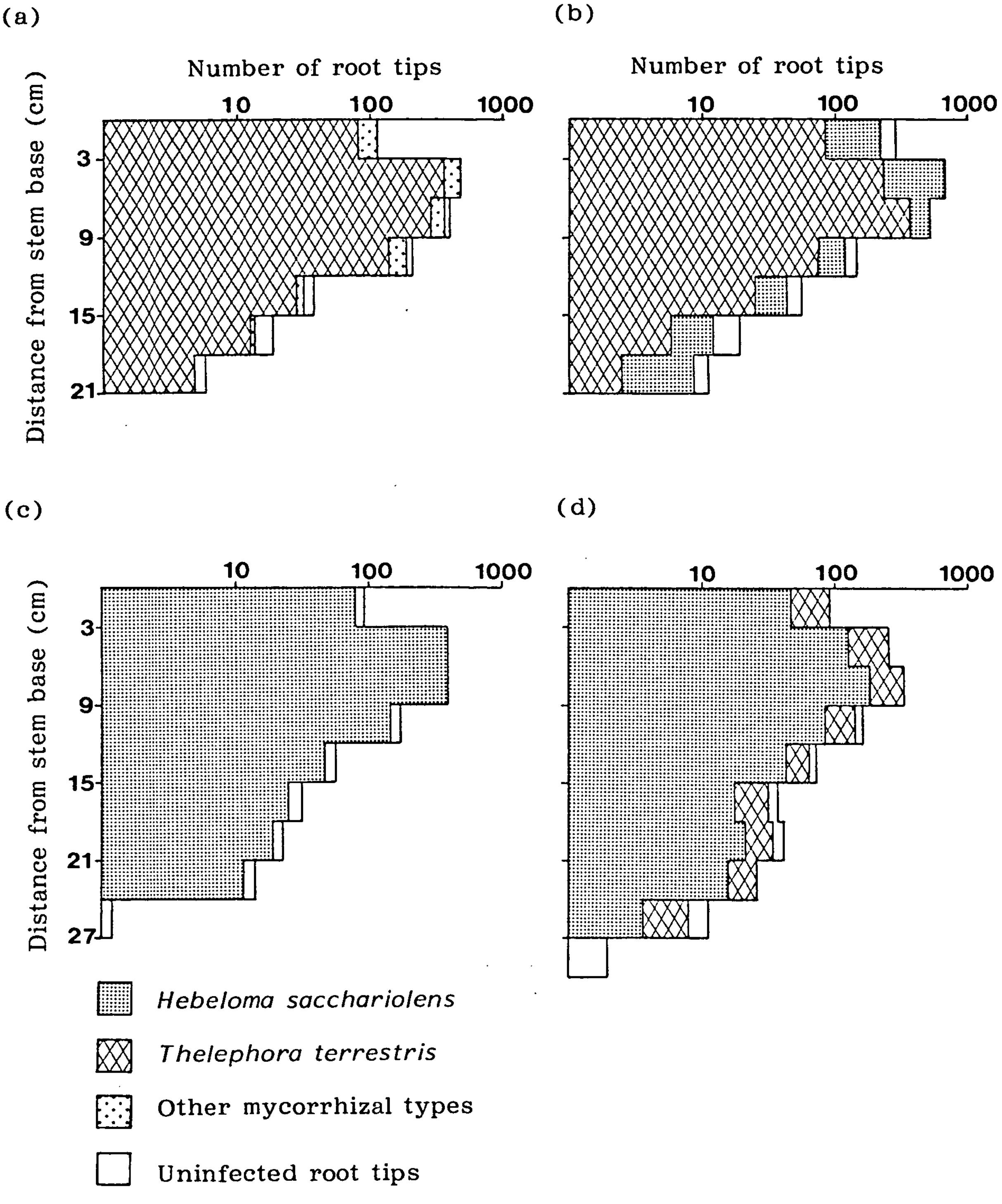

FIGURE 4.3.2: Distribution, after 170 days, at different distances from the stem base of seedlings (a) inoculated with *Thelephora terrestris* and transplanted into unsupplemented soil, (b) inoculated with *T. terrestris* and transplanted to soil supplemented with *Hebeloma sacchariolens*, (c) inoculated with *H. sacchariolens* and transplanted into unsupplemented soil, (d) inoculated with *H. sacchariolens* and transplanted to soil containing *T. terrestris*.

PLATE 4.2: Mycorrhizas of *Paxillus involutus* (arrowed) and mycorrhizas of *Hebeloma sacchariolens* with white sclerotium-like bodies, occurring on a single birch root.

the degree of colonisation of the seedlings by *H. sacchariolens* or *T. terrestris* was reduced by the presence of the other fungus of this pair.

4.3.2 Competitive Interactions Between Three Mycorrhizal Fungi in Brown Earth and Peat

In this experiment, similar to the previous one, the competitive interactions between three mycorrhizal fungi were studied in two types of soil. A different isolate of *P. involutus* was used than that previously, in case the results of the last experiment were due to deficiency of the isolate, and the combination of any single fungus as primary and secondary inoculants was omitted as unnecessary.

Aseptically germinated birch seedlings were transferred on 2 November 1982 to propagators containing a mixture of vermiculite-peat and inoculum of (1) *H. sacchariolens* (isolate 4), (2) *T. terrestris* (isolate 1) or (3) *P. involutus* (isolate 32); uninoculated vermiculite-peat was used as a fourth treatment (Section 2.3.2). The propagators were then incubated in a growth room and, because of the poor growth of some seedlings, each plant was given 4 cm^3 of Ingestad's solution (6.5 $mg\,l^{-1}$ phosphate; Mason, 1980) on 19 January 1983. 'Non-mycorrhizal' Bush soil was collected on 21 February 1983 and at the same time a commercially supplied sphagnum peat was thoroughly soaked in water. These 'soils' were mixed, separately, with the vermiculite-peat inocula of the three fungi, or with autoclaved inoculum (3 : 1, v/v); these supplements slightly raised the pH, from 3.9 to 4.0 (peat) or from 5.8 to 5.9 (brown earth). Plastic plant pots (75 mm diameter) were filled with the soils and a seedling was transplanted into each pot on 23 February 1983, when the seedlings were 114 days old. Four replicate randomised blocks were prepared, each containing factorial combinations of seedling and soil inoculation treatments but excluding 'like into like'. The pots were incubated in a heated glasshouse (max. 19.8°C, min. 13.7°C) for 77 days and then harvested (9 May 1983). The shoot heights and dry weights and the numbers of mycorrhizal tips were recorded, the terms primary and secondary inoculant being used as before (Section 4.3.1).

At the time of transplanting, the non-mycorrhizal (control) seedlings were largest in terms of height and dry weight (Table 4.3.8) and they also bore most root tips. All the inoculated seedlings bore mycorrhizas of the inoculant fungi.

After 77 days from transplanting, seedlings in peat were both taller and heavier than their counterparts in Bush soil (Table 4.3.9). Because of this, growth analyses for the two 'soils' were done separately and, for ease of handling, were based on pooled results for primary or secondary inoculant treatments as appropriate (Tables 4.3.10 and 4.3.11). The control seedlings were still the tallest and heaviest in Bush soil but not always significantly so, while seedlings inoculated initially with *P. involutus* were the smallest in Bush soil (Table 4.3.10); there was little difference between treatments in seedling growth in peat. As secondary inocula, none of the fungi had a significant effect on the shoot growth of the seedlings in either soil (Table 4.3.11).

Although initially established on seedlings, as a primary inoculant, *P. involutus* was present on very few seedlings after 77 days in soil of either type (Table 4.3.12). Yet, remarkably, *P. involutus* colonised all of the seedlings, irrespective of primary inoculum, when it was present as a secondary inoculant in the soils. *T. terrestris*, by contrast, persisted on nearly all of the seedlings when used as a primary inoculant. Again, this behaviour occurred irrespective of soil type. But

TABLE 4.3.8: Mean (± s.e.) shoot height and dry weight and numbers of root tips on seedlings incubated for 114 days with inoculum of three mycorrhizal fungi.

	Seedling inoculation			
	Paxillus involutus (n=5)	*Thelephora terrestris* (n=5)	*Hebeloma sacchariolens* (n=4)	None (n=3)
Height (mm)	16 ± 1.1	21 ± 1.1	22 ± 1.6	35 ± 4.3
Dry weight (mg)	2.5 ± 0.33	3.7 ± 0.38	4.0 ± 0.38	21.2± 5.32
Mycorrhizal root tips	8 ± 0.7	15 ± 3.0	4 ± 1.6	0
Uninfected root tips	14 ± 1.1	14 ± 2.0	9 ± 1.7	109 ± 10.1
Total root tips	22 ± 1.2	29 ± 3.4	13 ± 1.3	109 ± 10.1

TABLE 4.3.9: Mean height and oven-dry weight of seedlings (pooled results for all inoculation treatments) grown in brown earth or peat for 77 days; data transformed to log(x+1) for analysis (in parentheses).

	Soil		L.S.D. (p = 0.05)
	Brown earth	Peat	
Height (mm)	27 *(1.431)*	64 *(1.798)*	*(0.042)*
Dry weight (mg)	25 *(1.363)*	195 *(2.252)*	*(0.089)*

TABLE 4.3.10: Mean shoot height and oven-dry weight of seedlings initially propagated with three mycorrhizal fungi and then grown for 77 days in brown earth or peat supplemented with inoculum of mycorrhizal fungi; data pooled for soil supplementation treatments and transformed to log(x+1) for analysis (in parentheses)*.

Soil		Seedling inoculant			
		Paxillus involutus	*Thelephora terrestris*	*Hebeloma sacchariolens*	None
Brown earth	Height (mm)	21^{ab} *(1.334)*	26 *(1.425)*	28^{a} *(1.460)*	31^{b} *(1.499)*
	Dry weight (mg)	14.8^{abc} *(1.160)*	21.1^{ad} *(1.354)*	25.1^{b} *(1.396)*	34.7^{cd} *(1.529)*
Peat	Height (mm)	60 *(1.774)*	57^{a} *(1.747)*	74^{a} *(1.871)*	63 *(1.801)*
	Dry weight (mg)	181.9 *(2.229)*	151.2^{ab} *(2.085)*	236.0^{a} *(2.369)*	208.8^{b} *(2.315)*

* Figures in each row followed by the same letter differ from one another at $p = 0.05$.

TABLE 4.3.11: Mean shoot height and oven-dry weight of seedlings initially propagated with three mycorrhizal fungi and then grown for 77 days in brown earth or peat supplemented with inoculum of mycorrhizal fungi; data pooled for seedling inoculation treatments and transformed to log(x+1) for analysis (in parentheses)*.

Soil		Soil inoculum addition			
		Paxillus involutus	*Thelephora terrestris*	*Hebeloma sacchariolens*	None
Brown earth	Height (mm)	27 *(1.435)*	26 *(1.458)*	25 *(1.403)*	27 *(1.436)*
	Dry weight (mg)	29.2 *(1.448)*	23.4 *(1.349)*	18.3 *(1.263)*	26.3 *(1.378)*
Peat	Height (mm)	63 *(1.803)*	72 *(1.858)*	58 *(1.756)*	61 *(1.788)*
	Dry weight (mg)	201.7 *(2.281)*	243.5 *(2.381)*	167.0 *(2.110)*	176.1 *(2.239)*

* There was found, by analysis of variance, to be no significant difference between treatments.

TABLE 4.3.12: Numbers of seedlings colonised by primary inoculant* type (x/-) and secondary inoculant* type (-/x) mycorrhizas after being incubated in brown earth or peat for 77 days (max. 4 replicate seedlings).

		Seedling (primary) inoculant			
Soil	Soil (secondary) inoculant	*Paxillus involutus*	*Thelephora terrestris*	*Hebeloma sacchariolens*	None
Brown earth	*P. involutus*	-	3/4	4/4	-/3**
	T. terrestris	1/4	-	4/1	-/3**
	H. sacchariolens	1/4	2/3**	-	-/3**
	None	1/-	3/-	4/-	-
Peat	*P. involutus*	-	3/4	0/4	-/4
	T. terrestris	0/4	-	3/4	-/3**
	H. sacchariolens	1/0	3/0	-	-/0
	None	0/-	4/-	3/-	-

* For explanation see text; ** Maximum of three replicates only.

H. sacchariolens showed a distinct difference in behaviour between the two soils: in Bush soil it persisted as a primary inoculant and it colonised all seedlings as a secondary inoculant, whereas in peat it persisted less well as a primary inoculant and did not colonise roots as a secondary inoculant.

Most of the general comments above are reinforced by data on the numbers of mycorrhizas of different types, presented in Tables 4.3.13 and 4.3.14. For example, the mycorrhizas of *P. involutus* were never dominant when present from seedling-borne inoculum but were always dominant when they developed from soil-borne inoculum. *T. terrestris* was sometimes dominant as a primary inoculant, in either soil, and was always dominant as a secondary inoculant except where *H. sacchariolens* was present as a primary inoculant in Bush soil. *H. sacchariolens* was never dominant in the peat but was often dominant in Bush soil as a primary or secondary inoculant. The majority of the mycorrhizas grouped together as 'others' in Tables 4.3.13 and 4.3.14 were attributable to Thelephora-type in the peat and to Inocybe-type in Bush soil. The identification of these mycorrhizas was confirmed by the appearance of fruitbodies of *T. terrestris* and *Inocybe lacera* in some of the pots early in May.

In summary, this experiment demonstrates, for a different isolate than before, that *P. involutus* does not readily colonise seedling roots from seedling-borne inoculum after transplanting; rather, in many cases this fungus was completely replaced on the root systems. However, this same isolate heavily colonised seedlings from soil-borne inoculum, even in Bush soil, to such an extent that it was always dominant. Perhaps, *P. involutus* depends more on recurrent infections from the soil than on ectotrophic growth along roots in order to establish

TABLE 4.3.13: Mean numbers (± s.e.) of primary and secondary inoculant* type mycorrhizas and other mycorrhizal types on seedlings grown in brown earth (Bush soil) for 77 days.

Soil (secondary) inoculant	Mycorrhizal types present**	Seedling (primary) inoculant			
		Paxillus involutus	*Thelephora terrestris*	*Hebeloma sacchariolens*	None
Paxillus involutus	1°	-	31 ± 17.6	54 ± 20.8	-
	2°	-	165 ± 47.9	199 ± 64.9	302 ± 128.4
	others	-	0	0	92 ± 45.9
	none	-	28 ± 12.5	38 ± 3.4	47 ± 12.0
Thelephora terrestris	1°	15 ± 14.7	-	100 ± 26.5	-
	2°	121 ± 40.0	-	1 ± 0.7	80 ± 15.8
	others	5 ± 3.5	-	0	99 ± 39.8
	none	140 ± 60.9	-	28 ± 4.6	59 ± 14.2
Hebeloma sacchariolens	1°	26 ± 26.5	10 ± 5.2	-	-
	2°	89 ± 15.9	107 ± 12.9	-	98 ± 18.7
	others	0	0	-	82 ± 20.4
	none	25 ± 7.8	31 ± 5.9	-	43 ± 10.2
None	1°	13 ± 2.9	140 ± 58.8	155 ± 25.8	-
	2°	-	-	-	-
	others	3 ± 2.7	20 ± 14.4	5 ± 1.1	214 ± 20.3
	none	82 ± 21.0	129 ± 35.5	36 ± 11.6	87 ± 6.9

* For explanation see text.

** 1°, primary inoculant type mycorrhizas; 2°, secondary inoculant type mycorrhizas; others, other mycorrhizal types; none, uninfected root tips.

TABLE 4.3.14: Mean numbers (± s.e.) of primary and secondary inoculant* type mycorrhizas and other mycorrhizal types on seedlings grown in peat for 77 days.

Soil (secondary) inoculant	Mycorrhizal types present**	Seedling (primary) inoculant			
		Paxillus involutus	*Thelephora terrestris*	*Hebeloma sacchariolens*	None
Paxillus involutus	1°	-	81 ± 39.1	0	-
	2°	-	288 ± 83.7	366 ± 88.0	601 ± 77.7
	others	-	0	8 ± 7.7	53 ± 18.9
	none	-	82 ± 16.5	161 ± 56.5	209 ± 39.0
Thelephora terrestris	1°	0	-	4 ± 2.1	-
	2°	419 ± 84.5	-	450 ± 58.8	545 ± 8.8
	others	0	-	0	0
	none	174 ± 38.0	-	173 ± 15.4	282 ± 39.4
Hebeloma sacchariolens	1°	1 ± 0.7	201 ± 123.4	-	-
	2°	0	0	-	0
	others	143 ± 100.2	0	-	176 ± 58.5
	none	381 ± 115.1	146 ± 54.3	-	773 ± 100.8
None	1°	0	333 ± 8.1	11 ± 4.1	-
	2°	-	-	-	-
	others	264 ± 51.9	0	292 ± 102.5	285 ± 97.0
	none	362 ± 87.6	74 ± 11.1	529 ± 85.5	598 ± 203.5

* For explanation see text.

** 1°, primary inoculant type mycorrhizas; 2°, secondary inoculant type mycorrhizas; others, other mycorrhizal types; none, uninfected root tips.

mycorrhizas. The ability of *H. sacchariolens* to colonise new roots from seedling- or soil-borne inoculum was markedly affected by soil type: in peat no new mycorrhizas were formed by this fungus whereas in Bush soil this mycorrhizal fungus was dominant as a primary or secondary inoculant unless *P. involutus* was present, when colonisation of roots by *H. sacchariolens* was depressed. The colonisation of roots by *T. terrestris* was not affected by soil type. But its colonisation of roots from seedling-borne inoculum was limited by soil-borne inoculum of *P. involutus*, and in Bush soil its colonisation of roots from soil-borne inoculum was reduced by *H. sacchariolens* already established on the seedlings.

4.4 Persistence of Mycorrhizas of *Lactarius pubescens* on Seedling Roots after Transplanting

Lactarius pubescens was considered to be a 'late-stage' fungus by Deacon *et al.* (1983), but by their definition the term 'late-stage' refers only to establishment of infection on seedlings in unsterile soil and does not take account of spread from pre-existing infections. It was, therefore, of interest to see if *L. pubescens*, once established, can persist and colonise newly emerging roots in soil both in the presence and in the absence of soil inoculum of other fungi. This experiment was done to investigate this possibility.

Birch seedlings were incubated in propagators as before (Section 2.3.2) containing either uninoculated, and therefore sterile vermiculite-peat or vermiculite-peat that had been inoculated with *L. pubescens* (isolate 6). Meanwhile, on 10 June 1982, 'non-mycorrhizal' Bush soil was collected and mixed with vermiculite-peat inoculum of *Hebeloma sacchariolens* (isolate 4), *Thelephora terrestris* (isolate 1) or inoculum that had been sterilised by autoclaving (3 : 1 v/v), and a fourth batch of soil was not supplemented with inoculum. For comparison with these, a further collection, of 'mycorrhizal' soil, was taken from beneath a stand of 10-year-old birch trees on the Bush Estate. On 12 June 1982, when 59 days old, the seedlings were transplanted into these soils in 75 mm diameter plastic plant pots; the two propagation treatments were combined factorially with all soil treatments, with ten-fold replication, and the pots were arranged in randomised blocks in a heated glasshouse (max. 28°C, min. 14°C). Five blocks were randomly selected and harvested after 79 days' growth (30 August 1982); the remaining blocks were sampled after 147 days' growth (6 November 1982). At each sampling the height and dry weight of the shoots were recorded, and the root systems were cut into 3 cm pieces, starting at the base of the stem, and assessed for mycorrhizal development.

At the time of transplanting the control seedlings were significantly taller but significantly lighter than the seedlings inoculated with *L. pubescens* (Table 4.4.1); there was no significant difference between the two treatments in the total number of root tips. The control seedlings remained the taller plants throughout the experiment but, at both sampling times, there was no significant difference in the shoot dry weights between the two seedling propagation treatments (Table 4.4.2). Similarly there was no significant difference in shoot dry weight or, generally, in shoot height between the seedlings grown in the different soil treatments (Table 4.4.3); inspection of the full results (not presented) showed no evidence of interaction between treatments in these respects.

Although at the time of transplanting, all of the seedlings inoculated with *L. pubescens* were observed to bear mycorrhizas of this fungus,

TABLE 4.4.1: Mean shoot heights and oven-dry weights and mean numbers of root tips on seedlings propagated for 59 days with *Lactarius pubescens* or no inoculum (control); data transformed to log(x+1) for analysis (in parentheses).

	Seedling inoculation		
	Lactarius pubescens	None	L.S.D. (p = 0.05)
Height (mm)	21 *(1.330)*	41 *(1.619)*	*(0.129)*
Dry weight (mg)	4.9 *(0.759)*	2.6 *(0.547)*	*(0.127)*
Mycorrhizal tips	13	0	
Total root tips	32 *(1.505)*	37 *(1.574)*	*(0.140)*

TABLE 4.4.2: Mean shoot heights and oven-dry weights of seedlings, at two sampling times, that were initially inoculated with *Lactarius pubescens* or uninoculated and transplanted to soils containing different mycorrhizal inocula; data pooled for different soil supplements and transformed to log(x+1) for analysis (in parentheses).

Sampling time		Seedling inoculation		L.S.D. (p = 0.05)
		L. pubescens	None	
79 days	Height (mm)	38 *(1.575)*	63 *(1.788)*	*(0.075)*
	Dry weight (mg)	71.9 *(1.816)*	92.5 *(1.903)*	*(0.151)*
147 days	Height (mm)	57 *(1.755)*	69 *(1.833)*	*(0.053)*
	Dry weight (mg)	154.9 *(2.131)*	161.9 *(2.203)*	*(0.133)*

TABLE 4.4.3: Mean shoot heights and oven-dry weights of seedlings, at two sampling times, that were initially inoculated with *Lactarius pubescens* or uninoculated and transplanted to soils containing different mycorrhizal inocula; data pooled for seedling propagation treatments and transformed to log(x+1) for analysis (in parentheses).

Sampling time		Soil inoculum					L.S.D. (p= 0.05)
		Hebeloma sacchariolens	*Thelephora terrestris*	Sterile inoculum	Mycorrhizal soil	Non-mycorrhizal soil	
79 days	Height (mm)	53 *(1.705)*	43 *(1.657)*	57 *(1.741)*	47 *(1.633)*	54 *(1.672)*	*(0.118)*
	Dry weight (mg)	68.9 *(1.780)*	62.1 *(1.821)*	102.7 *(1.952)*	77.3 *(1.849)*	104.2 *(1.896)*	*(0.239)*
147 days	Height (mm)	60 *(1.795)*	56 *(1.731)*	64 *(1.798)*	64 *(1.808)*	71 *(1.839)*	*(0.083)*
	Dry weight (mg)	124.1 *(2.079)*	156.3 *(2.156)*	156.9 *(2.186)*	177.9 *(2.247)*	173.4 *(2.168)*	*(0.211)*

yet after 79 days the fungus appeared to have died out on many of the root systems (Table 4.4.4). The numbers of Lactarius-type mycorrhizas on the seedlings were, in general, low (Table 4.4.5) and represented only 10.7% of the total number of root tips. After 147 days, however, nearly all of the inoculated seedlings bore Lactarius-type mycorrhizas (Table 4.4.4), with larger mean numbers of this mycorrhizal type than before on individual seedlings (Table 4.4.6); at this sampling *L. pubescens* occupied 28.6% of all root tips. This difference between samplings, although not easily explained, is unlikely to have been due to sampling error. In fact, Lactarius-type mycorrhizas were also seen on the initially non-mycorrhizal (control) seedlings at 147 days (Tables 4.4.4 and 4.4.6) though only 2.2% of their root tips were attributable to Lactarius-type mycorrhizas. There was no effect of soil treatment on the mean numbers of Lactarius-type mycorrhizas on the seedlings (Table 4.4.5 and 4.4.6), mainly because of the large degree of variation between replicates.

Even though *L. pubescens* was still present on seedling roots at the end of the experiment and was occupying an increasing proportion of root tips, the majority of mycorrhizas on the seedlings were formed by fungi other than *L. pubescens* (Tables 4.4.5 and 4.4.6). In the soils supplemented with inoculum of *T. terrestris* or *H. sacchariolens*, the mycorrhizas formed on the seedlings, except for those of *L. pubescens*, were almost exclusively formed by the two secondary inoculants; in this respect the mycorrhizas of *H. sacchariolens* could be distinguished from those of other *Hebeloma* spp. by the presence of conspicuous white sclerotium-like bodies. Seedlings that were grown in unsupplemented soils or in soil supplemented with sterile inoculum, were heavily colonised from naturally occurring inoculum, the mycorrhizas being termed 'others'

TABLE 4.4.4: Numbers of seedlings (max. 5) infected by *Lactarius pubescens* at two sampling times; seedlings initially inoculated with *L. pubescens* or uninoculated (control) and transplanted into soil supplemented with different mycorrhizal inoculum.

		Soil inoculum				
Sampling time	Seedling inoculation	*Hebeloma sacchariolens*	*Thelephora terrestris*	Sterile inoculum	Mycorrhizal soil	Non-mycorrhizal soil
79 days	*L. pubescens*	4	1	3	2	2*
	Control	0	0	0	0	0
147 days	*L. pubescens*	4	4*	4*	5	5
	Control	0*	3	0*	3	0

* maximum of 4 seedlings only.

TABLE 4.4.5: Mean numbers (± s.e.) of root tips of different mycorrhizal types on seedlings either inoculated with *Lactarius pubescens* or uninoculated and grown for 79 days in soil supplemented with the inoculum of mycorrhizal fungi (termed secondary inoculant)*.

Soil inoculum addition (secondary inoculant)	*Lactarius pubescens*				None			
	Lactarius pubescens	Secondary inoculant	Other types	Total root tips	*Lactarius pubescens*	Secondary inoculant	Other types	Total root tips
Hebeloma sacchariolens	13 ± 6.8	201 ± 56.0	7 ± 7.4	277 ± 69.2	0	213 ± 32.4	1 ± 0.8	252 ± 37.3
Thelephora terrestris	tr**	150 ± 27.5	1 ± 1.0	193 ± 35.9	0	321 ± 40.0	0	376 ± 42.4
Sterile inoculum	105 ± 52.4	0	234 ± 96.8	502 ± 74.6	0	0	310 ± 43.8	501 ± 55.8
Mycorrhizal soil	3 ± 2.1	0	256 ± 64.8	343 ± 72.6	0	0	230 ± 98.3	309 ± 109.5
Non-mycorrhizal soil	74 ± 56.6	0	218 ± 92.2	455 ± 150.8	0	0	380 ± 75.3	571 ± 105.6
Mean	*37 ± 15.8*	*73 ± 21.9*	*131 ± 35.8*	*350 ± 40.8*	*0*	*107 ± 29.2*	*184 ± 40.1*	*402 ± 39.5*

* There was found, by analysis of variance of transformed data, to be no significant difference in the numbers of Lactarius-type mycorrhizas between treatments.

** Mean of <1 mycorrhizal tip of *L. pubescens* per seedling.

TABLE 4.4.6: Mean numbers (± s.e.) of root tips of different mycorrhizal types on seedlings either inoculated with *Lactarius pubescens* or uninoculated and grown for 147 days in soil supplemented with the inoculum of other mycorrhizal fungi (termed secondary inoculants)*.

Soil inoculum addition	*Lactarius pubescens*				None			
	Lactarius pubescens	Secondary inoculant	Other types	Total root tips	*Lactarius pubescens*	Secondary inoculant	Other types	Total root tips
Hebeloma sacchariolens	219 ± 94.4	279 ± 48.6	0	558 ± 23.8	0	493 ± 70.4	0	598 ± 86.6
Thelephora terrestris	54 ± 33.4	437 ± 39.1	0	668 ± 110.8	16 ± 6.9	309 ± 28.6	0	514 ± 21.5
Sterile inoculum	211 ± 90.9	0	365 ± 90.1	679 ± 119.2	0	0	550 ± 59.6	698 ± 100.0
Mycorrhizal soil	93 ± 42.2	0	433 ± 101.0	581 ± 85.4	50 ± 44.7	0	601 ± 68.9	748 ± 49.5
Non-mycorrhizal soil	295 ± 169.2	0	297 ± 74.9	647 ± 209.3	0	0	516 ± 51.7	611 ± 70.2
Mean	*178 ± 46.1*	*136 ± 39.5*	*222 ± 48.5*	*623 ± 53.1*	*14 ± 9.9*	*153 ± 44.2*	*338 ± 61.4*	*633 ± 32.3*

* There was found, by analysis of variance of transformed data, to be no significant difference in the numbers of Lactarius-type mycorrhizas between treatments.

in Tables 4.4.5 and 4.4.6. Predominant amongst these were the mycorrhizas of *T. terrestris* but others were attributable to *Hebeloma* sp., *Inocybe* sp. and *Laccaria* sp. Two mycorrhizal types, namely type 2 ('brown with cream tips') and type 6, were observed on seedlings grown in naturally 'mycorrhizal' soil, but not in the other soils.

The distributions of Lactarius-type mycorrhizas on the root systems are shown in Figures 4.4.1 and 4.4.2. The initial depth of the soil 'plug' that had been transplanted was 6 cm and it can be seen from Figure 4.4.1 that even by the first sampling date *L. pubescens* had spread from this initially colonised region. After 147 days Lactarius-type mycorrhizas were found at a linear distance of 24 cm down the root systems from the base of the stem, although the furthest roots by this time were up to 39 cm from the stem base (Figure 4.4.2). However, the rooting depth of the pots was only 7 cm and consequently the roots must have been coiling around inside the pot; this would have had the effect of bringing actively growing root tips into close proximity to established mycorrhizas, from which the new tips may have been infected. Interestingly, although natural infection of the control seedlings by *L. pubescens* had occurred by 147 days the mycorrhizas of *L. pubescens* were all found within 12 cm distance of the stem base - that is, in the older parts of the root system.

In summary, this experiment has demonstrated that an isolate of *L. pubescens* established on seedling root systems, could persist and spread on the roots after transplanting into soil, irrespective of the presence of the inoculum of other mycorrhizal fungi. However, it did not markedly affect colonisation by these other mycorrhizal fungi, perhaps because in this experiment *L. pubescens* was present on only a minor proportion of the root system.

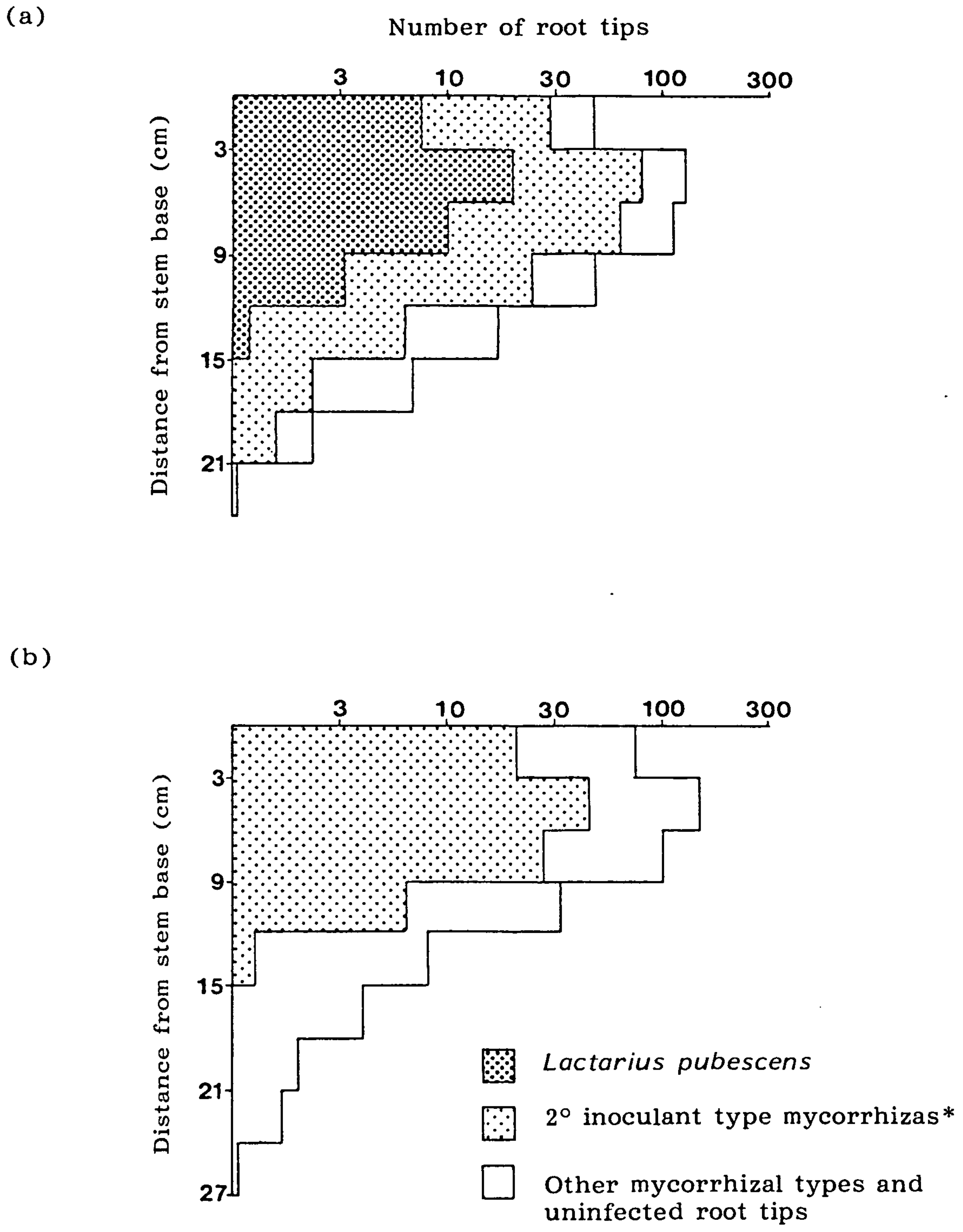

FIGURE 4.4.1: Distribution of mycorrhizas down the root systems of birch seedlings either inoculated with (a) *Lactarius pubescens* or (b) uninoculated and grown in brown earth for 79 days.

* For explanation see text.

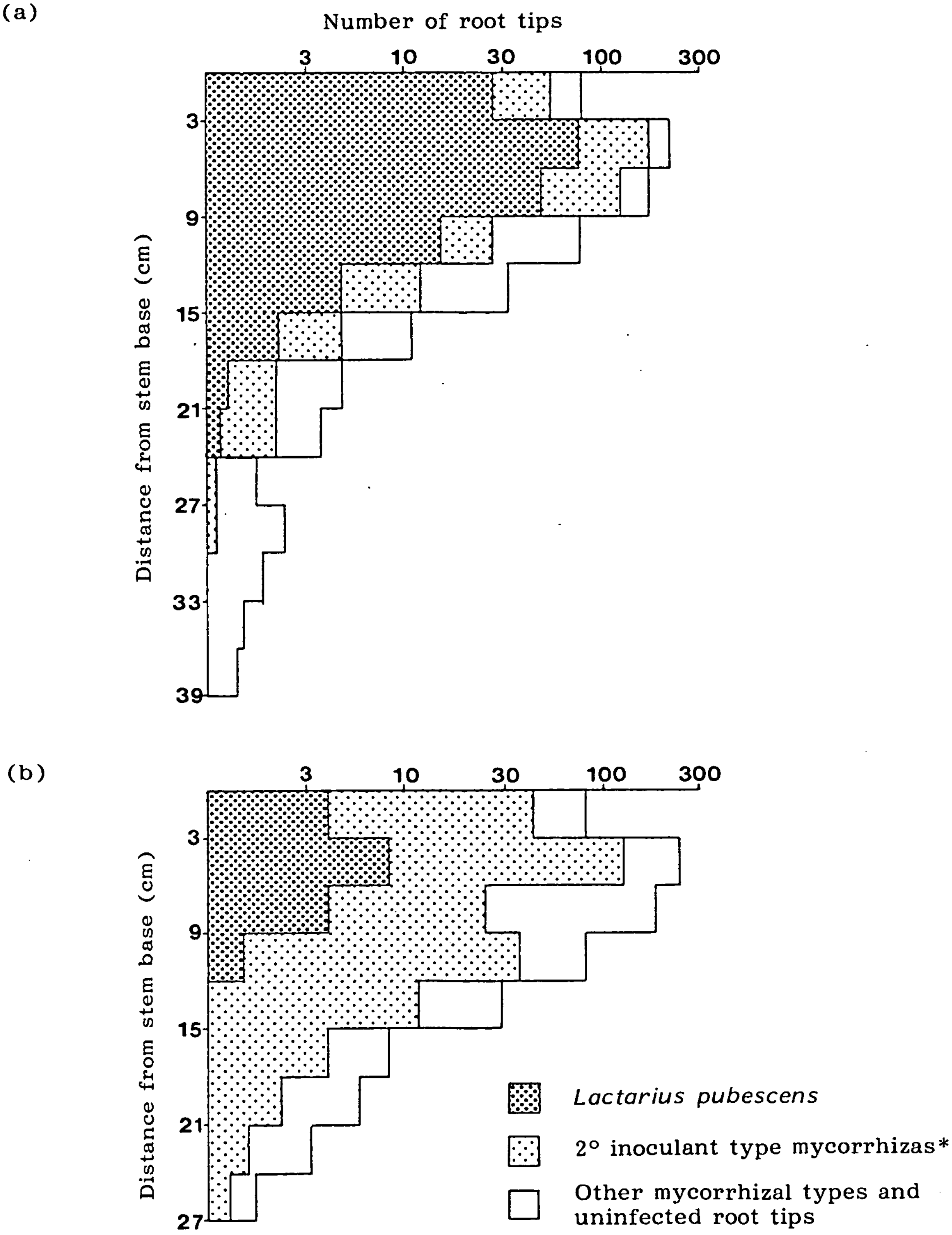

FIGURE 4.4.2: Distribution of mycorrhizas down the root systems of birch seedlings either inoculated with (a) *Lactarius pubescens* or (b) uninoculated and grown in brown earth for 147 days.

* For explanation see text.

In a similar experiment, not reported here because it is not yet complete, seedlings propagated with *L. pubescens* were transplanted to brown earth. After 215 days' growth Lactarius-type mycorrhizas were observed on only 3 of 6 seedlings sampled and accounted for only 3.1% of root tips overall; the majority of mycorrhizas on the seedlings were formed by naturally occurring types.

4.5 Persistence of 'Late-Stage' Mycorrhizal Fungi on Seedlings After Transplanting

Mason *et al.* (1983a) reported that even when *Amanita muscaria* was established on seedling roots in bixenic culture, it would not colonise new roots after the seedlings were transferred to unsterile soil. Furthermore, after a period of time the original mycorrhizas could no longer be observed on the transplanted root system. The following three experiments were done to see if this behaviour is typical of 'late-stage' mycorrhizal fungi and if it can be affected by manipulating soil conditions.

4.5.1 Persistence of the Mycorrhizas of Amanita muscaria and Leccinum scabrum in Sterilised and Unsterile Soil

It was considered possible that competition from resident mycorrhizal inoculum or from other members of the soil microflora may affect the ability of some 'late-stage' mycorrhizal fungi to colonise new roots in soil. Steam-sterilisation is known to destroy both soil-borne pathogens and potentially antagonistic saprophytes (Olsen and Baker, 1968), so this procedure was now used to reduce potential competition or antagonism against 'late-stage' mycorrhizal fungi established on seedlings transplanted to soil.

'Non-mycorrhizal' Bush soil was collected on 5 February 1981 and stored in trays at room temperature until friable enough to pass through a 1 cm^2 sieve; the soil was not allowed completely to air-dry. Half of the soil was sterilised by steaming in an autoclave at 110°C for 20 minutes; the remainder was left untreated. Meanwhile, seedlings were grown in propagators in sterilised vermiculite-peat (Section 2.3.2) either without inoculum or inoculated with *A. muscaria* (isolate 71) or *Leccinum scabrum* (isolate 6). The seedlings were incubated in a growth cabinet for 82 days when they were ready for transplanting (9 February 1982); a sample of five seedlings was taken from each propagator at this time and assessed for shoot growth and mycorrhizal infection. Plant pots, 75 mm diameter, were filled with steamed or untreated soil, and the seedlings were transplanted into them on 9 February 1982, giving six separate treatments (2 soils x 3 seedling propagation treatments). The experiment, with eight-fold replication, was arranged as eight randomised blocks in a heated glasshouse (max. 24°C, min 14°C). Four blocks were randomly selected and sampled after 60 days (10 April 1982); the remaining blocks were incubated for a further 75 days before being sampled (24 June 1982).

At the time of transplanting there was little difference in the height or total number of root tips between seedlings in different propagation treatments, although the non-mycorrhizal control plants had heavier shoots than had the inoculated plants (Table 4.5.1). All of the inoculated plants had Leccinum-type or Amanita-type mycorrhizas. After 60 and 135 days growth in a glasshouse there was no significant effect of seedling inoculation on the height or dry weight of the seedling shoots (Table 4.5.2). However, at both sampling times the seedlings grown in the sterilised soil were significantly taller and heavier than

TABLE 4.5.1: Mean shoot height and oven-dry weight and mean number of root tips on seedlings incubated for 82 days with *Amanita muscaria*, *Leccinum scabrum* or no inoculum (n=5).

Inoculant fungus	Height (mm)	Dry weight (mg)	Inoculant type mycorrhizas	Total root tips
Amanita muscaria	24 ± 4.4	3.0 ± 0.43	27 ± 7.2	73 ± 6.5
Leccinum scabrum	22 ± 1.2	2.3 ± 0.20	23 ± 2.2	67 ± 6.4
None	25 ± 2.7	4.8 ± 0.76	0	70 ± 9.4

TABLE 4.5.2: Mean shoot height and oven-dry weight of birch seedlings propagated in the presence of *Amanita muscaria* or *Leccinum scabrum* or in uninoculated vermiculite-peat and subsequently grown in steam-sterilised or unsterile soil for 60 or 135 days; data transformed to log(x+1) for analysis (in parentheses).

		Seedling inoculation (pooled data for soil treatments)			
Sampling time		*Amanita muscaria*	*Leccinum scabrum*	None	*L.S.D. (p =0. 05)*
60 days	Height (mm)	40 *(1.601)*	45 *(1.650)*	47 *(1.677)*	*(0.085)*
	Dry weight (mg)	43.3 *(1.570)*	37.8 *(1.530)*	49.1 *(1.641)*	*(0.268)*
135 days	Height (mm)	92 *(1.938)*	89 *(1.925)*	81 *(1.889)*	*(0.083)*
	Dry weight (mg)	319.4 *(2.443)*	313.2 *(2.431)*	295.4 *(2.397)*	*(0.129)*

		Soil treatment (pooled data for propagation treatments)		
		Sterilised	Unsterile	
60 days	Height (mm)	50 *(1.697)*	38 *(1.588)*	*(0.028)*
	Dry weight (mg)	52.8 *(1.687)*	33.9 *(1.474)*	*(0.154)*
135 days	Height (mm)	115 *(2.059)*	59 *(1.776)*	*(0.068)*
	Dry weight (mg)	454.9 *(2.643)*	163.8 *(2.204)*	*(0.106)*

their counterparts grown in the unsterile soil. The increased growth of seedlings in the sterilised soil probably results from a greater mineralisation of nutrients such as nitrogen during or following sterilisation (Powlson and Jenkinson, 1976).

After 60 days there was no difference in the numbers of Amanita-type or Leccinum-type mycorrhizas on the seedlings grown in sterilised or unsterile soil; therefore the results for both soil treatments are combined in the presented results (Table 4.5.3). Both mycorrhizal types had decreased in numbers since transplanting (c.f. Tables 4.5.1 and 4.5.3) and by the time of the second sampling no Leccinum-type or Amanita-type mycorrhizas were observed in any treatment. Although there was some colonisation of the seedlings after 60 days by naturally occurring mycorrhizal types, by far the majority of root tips were uninfected at this stage. However, at the second sampling time all of the seedlings were heavily colonised by 'naturally occurring' mycorrhizal types; these were formed mainly by *Hebeloma* sp., *Laccaria* sp., *Inocybe* sp. and *Thelephora terrestris*

The results from this experiment indicate that steam sterilisation has no effect on the persistence of two 'late-stage' mycorrhizal fungi, established on seedlings that were subsequently transplanted to the soil. The mycorrhizas of both fungi, *A. muscaria* and *L. scabrum*, declined in numbers until none was recognisable. All of the seedlings were ultimately infected by other mycorrhizal fungi; presumably, in the case of sterilised soil, infection was caused by air-borne inocula such as basidiospores.

TABLE 4.5.3: Mean numbers (± s.e.) of root tips of different mycorrhizal types on seedlings originally inoculated with different mycorrhizal fungi and grown for 60 or 135 days in soil in a glasshouse; data pooled for different soil treatments (n=8).

Sampling time	Inoculant fungus	Inoculant-type mycorrhizas	Other mycorrhizal types	Total root tips
60 days	*Amanita muscaria*	9 ± 2.1	4 ± 3.9	199 ± 27.4
	Leccinum scabrum	10 ± 3.6	5 ± 3.1	278 ± 44.0
	None	-	0	284 ± 47.3
135 days	*Amanita muscaria*	0	3222 ± 1018.9	3384 ± 1041.6
	Leccinum scabrum	0	2479 ± 638.5	3118 ± 691.4
	None	-	2439 ± 698.5	3068 ± 833.9

4.5.2 *Persistence of the Mycorrhizas of Amanita muscaria in Sterilised Soil Supplemented with Mycorrhizal Inoculum*

Competition from mycorrhizal fungi that are resident or introduced into soil may affect the ability of some established mycorrhizal fungi on seedlings to colonise new roots (Section 4.3). This experiment was done to see if spread of infection by *Amanita muscaria*, already established on seedling root systems, was influenced by the presence of inoculum of other mycorrhizal fungi introduced into the soil, the resident mycorrhizal inoculum having first been destroyed by sterilisation. The fungi added to the soil were *Thelephora terrestris* (isolate 1), *Lactarius pubescens* (isolate 6) and *A. muscaria* (isolate 71); the experiment thus provided a comparison with other experiments (Section 4.2.1) on the ability of *A. muscaria* and *L. pubescens* to colonise seedlings from soil-borne inoculum.

Soil was collected and sterilised as described before (Section 4.5.1); it was then mixed, separately, with vermiculite-peat inoculum of the three fungi or autoclaved inoculum in the ratio 3 : 1 (v/v). Seedlings inoculated with *A. muscaria* or uninoculated control seedlings were taken, after 83 days, from the propagators in the last experiment (Table 4.5.1) and transplanted, on 10 February 1982, into the four batches of soil in 75 mm diameter pots. The experiment with six-fold replication was arranged in a heated glasshouse (max. 24°C, min. 14°C). Three blocks were randomly selected and sampled on 17 April 1982, after 66 days' growth in soil (Table 4.5.4).

There was no significant difference in shoot height or dry weight between the two inoculation treatments at the 66-day sampling; however, growth of the seedlings in the soil supplemented with *T. terrestris* was markedly depressed compared with that in the other soil treatments.

TABLE 4.5.4: Mean shoot height and oven-dry weight of seedlings propagated with different mycorrhizal fungi and grown for 66 days in soil supplemented with different mycorrhizal inocula; data transformed to log(x+1) for analysis (in parentheses).

	Seedling inoculation (pooled data for soil treatments)		
	Amanita muscaria	None	L.S.D. (p= 0.05)
Height (mm)	71 *(1.830)*	73 *(1.851)*	*(0.084)*
Dry weight (mg)	199.8 *(2.175)*	148.9 *(2.084)*	*(0.255)*

	Soil supplementation (data pooled for propagation treatments)				
	Amanita muscaria	*Lactarius pubescens*	*Thelephora terrestris*	None	
Height (mm)	79 *(1.902)*	70 *(1.876)*	44 *(1.641)*	87 *(1.942)*	*(0.068)*
Dry weight (mg)	197.0 *(2.285)*	180.2 *(2.142)*	65.7 *(1.689)*	254.3 *(2.403)*	*(0.361)*

There were also many fewer root tips on these seedlings than on those grown in the other soils (Table 4.5.5). Mycorrhizas of *A. muscaria* could be found on only four seedlings, one in each soil treatment, initially inoculated with this fungus; moreover, there was a decline in the numbers of these mycorrhizas, both on individual seedlings and overall (cf. Tables 4.5.1 and 4.5.5). Supplementing the soil with inoculum of either *A. muscaria* or *L. pubescens* did not lead to the formation of mycorrhizas of these fungi; however, *T. terrestris* colonised the seedling roots from soil-borne inoculum in the appropriate treatment. Infection of seedlings by *T. terrestris* occurred also in the other soil treatments and may have been due to accidental transfer of inoculum from pot to pot during handling and incubation. No other mycorrhizal types were observed on the seedlings. On 19 May 1982 the fruitbodies of *T. terrestris* were observed in all, except one, of the remaining pots that had been supplemented with inoculum of this fungus. The remaining blocks were sampled at the beginning of June 1982, but no mycorrhizas of *A. muscaria* or *L. pubescens* were observed on any of the seedlings, so detailed assessments were not made.

This experiment again demonstrates that the mycorrhizas of *A. muscaria* do not survive for long periods on seedlings after they are transplanted to soil. Furthermore, the failure of *A. muscaria* to persist on seedlings does not seem to be due to competition from other mycorrhizal fungi because new mycorrhizas were not formed even when numerous uninfected root tips were available for colonisation. The experiment also confirms that *A. muscaria* and *L. pubescens* do not colonise seedlings from inoculum added to soil even when the soil has been steam-sterilised; *T. terrestris*, by contrast, readily does so.

TABLE 4.5.5: Mean numbers (± s.e.) of root tips of different mycorrhizal types on seedlings inoculated with *Amanita muscaria* or uninoculated and grown for 66 days in soil supplemented with the inoculum of different mycorrhizal fungi (n=3).

	Seedling inoculation				
	Amanita muscaria			None	
Soil inoculum supplement	Amanita-type mycorrhizas	Thelephora-type mycorrhizas	Total root tips	Thelephora-type mycorrhizas	Total root tips
Amanita muscaria	1 ± 1.3	0	1202 ± 201.1	1 ± 0.7	1143 ± 249.3
Lactarius pubescens	2 ± 2.3	12 ± 6.7	1102 ± 328.4	5 ± 4.7	788 ± 376.0
Thelephora terrestris	1 ± 1.0	338 ± 273.0	428 ± 300.3	54 ± 21.7	369 ± 52.9
None	7 ± 6.7	5 ± 4.4	1980 ± 293.9	12 ± 12.0	1858 ± 181.8

4.5.3 Persistence of the Mycorrhizas of Different Fungi on Seedlings Transplanted into Sterile Growth Medium

The mycorrhizas of *Amanita muscaria* and *Leccinum scabrum* formed during seedling propagation in aseptic conditions do not persist when the seedlings are transplanted into soil, even if this is steam-sterilised (Section 4.5.1). However, sterilised soils are rapidly re-colonised by soil micro-organisms that either survive the sterilisation treatment or arrive from external sources, and such organisms may include fungi and bacteria antagonistic to mycorrhizal fungi (Bowen and Theodorou, 1979). It was therefore decided to compare the spread of four mycorrhizal fungi, established on seedling roots in pure culture, on seedlings transplanted into a sterile growth medium. The medium (vermiculite-peat) contained mineral nutrients but no added carbon source.

Eighty transparent polystyrene sample bottles (8 x 2 cm diameter), each with a screw-cap pierced with a hole plugged with cotton wool, were sterilised by gamma irradiation (2.5 Mrad). Autoclaved vermiculite-peat medium (10-15 cm^3) supplemented with modified Melin-Norkrans solution (Section 2.1.3) was then aseptically transferred to each tube (Mason, 1980). The tubes were inoculated with *A. muscaria* (isolate 183), *Lactarius pubescens* (isolate 8), *Paxillus involutus* (isolate 32) or *Hebeloma sacchariolens* (isolate 4). The inocula were small volumes of vermiculite-peat cultures except in the case of *L. pubescens* which was introduced as three 1 cm diameter agar discs. Immediately after inoculation, an aseptically germinated birch seedling was added to each tube, and the tubes were then randomised and incubated for 101 days in a growth cabinet lined with aluminium-foil (Section 2.2.3). Meanwhile, wide-necked 500 cm^3 Erlenmeyer flasks were filled with 250 cm^3 of vermiculite-peat and supplemented with 180 cm^3 of modified Ingestad's solution containing 6.5 mgl^{-1} of phosphate (Mason, 1980); this concentration has been found to be optimal for the formation of mycorrhizas on birch on agar (P.A. Mason, personal communication). The flasks were plugged with cotton-wool bungs and autoclaved at 121°C for 30 minutes. Seedlings from each of the inoculation treatments were then transferred singly and aseptically to the flasks, care being taken to ensure that as little as possible of the original medium adhered to the transplanted roots and that the roots were covered with sterile medium to prevent drying out. The flasks were then randomised, with fifteen-fold replication, and placed in two growth cabinets. The five extra seedlings of the original twenty in the propagation tubes were destructively sampled and assessed for shoot growth and the numbers of mycorrhizal and uninfected root tips. After 84 days' incubation the seedlings in the flasks were sampled and the spread of mycorrhizal fungi along the root systems was assessed. At this stage some of the vermiculite-peat medium from each flask was transferred to a plate of half-strength potato-dextrose agar to check for possible contamination. Unfortunately, all of the seedlings originally inoculated with *A. muscaria* were contaminated and therefore discarded.

After 101 days' incubation in the propagation tubes there was no difference between treatments in shoot height or dry weight of the seedlings (Table 4.5.6). All except one of the seedlings examined bore mycorrhizas of the inoculant fungus. After 84 days' growth in the flasks the seedlings inoculated with *P. involutus* were the tallest and heaviest, but generally not significantly so (Table 4.5.7). Only the mycorrhizas of *P. involutus* showed a large, and significant, increase in numbers after incubation in the flasks (c.f. Tables 4.5.6 and 4.5.7) though there were slight increases also in the numbers of mycorrhizas of *L. pubescens*

TABLE 4.5.6: Mean (± s.e.) shoot height and oven-dry weight and mean numbers of root tips on seedlings propagated for 101 days in the presence of three mycorrhizal fungi (n=5).

	Height (mm)	Dry weight (mg)	No. inoculant type mycorrhizas	No. uninfected root tips	Total No. root tips
Paxillus involutus	19 ± 5.2	2.5 ± 1.19	15 ± 8.1	7 ± 2.1	22 ± 7.2
Lactarius pubescens	17 ± 1.3	1.3 ± 0.37	12 ± 4.5	8 ± 2.7	20 ± 4.3
Hebeloma sacchariolens	15 ± 1.6	1.7 ± 0.23	7 ± 2.7	10 ± 3.0	17 ± 3.7

TABLE 4.5.7: Shoot height and oven-dry weight and mean numbers of root tips on seedlings propagated in the presence of three mycorrhizal fungi and then transferred to a sterile growth medium for a further 84 days; means (± s.e.) for 15 replicates.

Inoculant fungus	Height (mm)	Dry weight (mg)	No. inoculant type mycorrhizas	No. uninfected root tips	Total No. root tips
*Paxillus involutus**	167 ± 12.2	113.1 ± 10.03	82 ± 21.0	142 ± 18.9	223 ± 17.9
Lactarius pubescens	122 ± 13.5	81.3 ± 15.79	16 ± 4.5	119 ± 16.3	135 ± 17.4
*Hebeloma sacchariolens**	153 ± 7.9	99.3 ± 10.63	14 ± 2.4	139 ± 13.8	152 ± 13.7

* 14 replicates only.

TABLE 4.5.8: Occurrence of contaminants and their effects on mycorrhizal development on seedlings propagated in the presence of three mycorrhizal fungi and transferred to sterile vermiculite-peat.

Inoculant fungus	Mean No. mycorrhizas after 84 days in flasks contaminated with different micro-organisms (No. flasks in each category in parentheses)		
	Not contaminated	Bacterial contaminants	Fungal contaminants
Paxillus involutus	31 ± 3.9 *(5)*	39 ± 10.3 *(7)*	6 ± 2.0 *(2)*
Lactarius pubescens	31 ± 4.0 *(2)*	14 ± 5.6 *(10)*	7 ± 3.6 *(3)*
Hebeloma sacchariolens	21 ± 21.3 *(1)*	9 ± 1.9 *(11)*	15 ± 7.4 *(2)*
Mean	34 ± 8.6 *(8)*	18 ± 4.0 *(28)*	8 ± 2.5 *(7)*

and *H. sacchariolens* in the respective treatments; in all treatments the proportion of root tips occupied by mycorrhizas was markedly lower at the end of the experiment than at the time of transplanting. Many of the flasks had bacterial or fungal contaminants though these were not visible by inspection alone, and Table 4.5.8 shows that the numbers of mycorrhizas in these contaminated flasks were generally lower than in non-contaminated flasks, the effect being most pronounced where bacterial contaminants were recorded.

The high degree of contamination in this experiment defeats its original purpose, yet it is clear that, even in the uncontaminated flasks, the mycorrhizal fungi colonised outgrowing roots of the seedlings very poorly. This would not have been expected for some of these fungi, so it seems that the design of the experiment was at fault. The experiment was not repeated because it would have entailed too much preliminary investigation into factors affecting mycorrhizal growth in pure culture, for which time was not available.

4.6 Influence of Propagating Soil on the Mycorrhizal Succession on Birch Seedlings Transplanted into a Field Site

This final experiment reported in Section 4 is part of a study initiated by S.J. Donaldson at the suggestion and under the direction of Dr. J.W. Deacon and Professor F.T. Last; it has been submitted for consideration by the editors of the Transactions of the British Mycological Society. My contribution to the work began in March 1981, when pots containing seedlings that had been propagated in different soils had been maintained for two growing seasons in an unheated, but frost-free, glasshouse with no supplementary lighting. Because the history and mycorrhizal status of the seedlings were known, it was decided to plant them out into a field site and then to sample them at intervals to determine how the initially established mycorrhizal associations persisted and affected subsequent colonisation by other mycorrhizal fungi.

Part of the paper, as submitted, is reproduced here with the qualification that it does not represent solely my work. Indeed, it could not have been done within the three years of my research programme.

4.6.1 Experimental

On 16 February 1979, two sets of soil cores 6 cm deep and 2 cm diameter were collected from field OS 127 at Bush Estate, Penicuik, Midlothian, where an experimental plot of 60 birch trees had been established in 1971 (Mason *et al.*, 1982). One set of cores - termed 'mycorrhizal' - was taken in the root zone about 60 cm from the base of a tree (*Betula pendula*, designated B26) that had supported fruitbodies of *Hebeloma crustuliniforme*, *Inocybe lanuginella* and *H. sacchariolens* in the previous year. The second set of cores - 'non-mycorrhizal' - was taken about 12 m beyond the edge of the experimental plot. Both sets of cores contained roots of grasses and herbs but only the 'mycorrhizal' set contained tree roots.

Some cores were destructively sampled to assess the mycorrhizal types in them. The others, 28 of each type, were wrapped individually in 'tubes' of polyethylene with open ends. They were inverted and sunk into moist sand in trays, which were placed beneath mercury vapour lamps in a glasshouse at 20°C. Each core was sown with five seeds of *B. pendula* lightly covered with sand, and the emerging seedlings were subsequently thinned to one per core. Their growth was assessed visually, and after 8 weeks from sowing (mid-April) 10 cores of each type were destructively sampled; shoot growth and the numbers and types of mycorrhizas were recorded.

At 8 weeks, the polyethylene was removed from the remaining 18 cores of each type and they were put individually into 12 cm square pots filled with non-mycorrhizal soil, a groundwater gley silt and clay of pH 6.4. The soil had been passed through a 3 mm sieve but was otherwise untreated. The pots were completely randomised in a glasshouse at 20°C but without supplementary lighting and they were watered as necessary. Periodically, the intact soil balls were carefully removed from the pots to assess the mycorrhizas visible on their sides and bases; afterwards the soil balls were replaced in pots with minimal disturbance.

The pots were kept in the glasshouse, without supplementary fertilisers, during 1979 and 1980, and the occurrence of fungal fruit-bodies was recorded as they appeared. Early in 1981, before bud-break, the seedlings were 'hardened-off' in a cold frame and they were transplanted into a field site on 20 March 1981 with their soil balls intact. The 34 surviving seedlings were completely randomised in a block, 6 x 6 with 2 gaps, and spaced 1.5 m apart within and between rows. The site was about 100 m away from the position where the soil cores had been taken originally and it had no history of tree growth. Trees were subsequently sampled from this site on 27 January 1982 (7 replicates) and 18 November 1982 (5 replicates); 5 replicates of each treatment remain. On sampling, the root systems were carefully dug from the soil and washed; the roots of each tree were then aligned and 1 cm long segments were cut with a razor at usually 10 cm intervals, starting at the base of the stem. The root segments were examined for numbers and types of mycorrhizas (Section 2.5.2c).

4.6.2 Results

Shoot growth:

Eight weeks after sowing, the shoots of seedlings in 'mycorrhizal' cores were significantly taller than in 'non-mycorrhizal' cores (2.1 compared with 1.4 ± 0.08 cm). The difference was still significant after 22 weeks (5.9 compared with 4.4 ± 0.30 cm) but it subsequently disappeared, associated with the development of mycorrhizas on all seedlings. At the final sampling, after 195 weeks, the respective shoot heights were 134 and 152 ± 8.0 cm. In the first year, but not in subsequent years, the 'mycorrhizal' seedlings went into dormancy earlier than did 'non-mycorrhizal' seedlings; by 12 November 1979, 13 out of 18 'mycorrhizal' plants were leafless, compared with only 5 of 18 'non-mycorrhizal' plants. However, there was no difference in time of vernal bud-break.

Mycorrhizas before outplanting:

The mycorrhizal soil cores taken from the field contained several mycorrhizal types, dominated by those that were brown with cream tips (BCT; type 2 mycorrhizas) possibly formed by a member of the Ascomycotina, but including also types attributable to *Inocybe* sp. and a few mycorrhizas attributable to *Hebeloma* sp.

After 8 weeks, seedlings in these 'mycorrhizal' cores had more total root tips (118 ± 17.6) and more mycorrhizas (78 ± 4.9) than did seedlings in 'non-mycorrhizal' cores (total tips, 60 ± 16.8; mycorrhizas, 5 ± 4.7). Most (68%) of the mycorrhizas in the former category were attributable to *Inocybe* sp. and 32% were BCT; the few mycorrhizas on seedlings in the 'non-mycorrhizal' cores were attributable to *Hebeloma* sp.

After 17 weeks' growth, in June 1979, the balance of mycorrhizal types seemed to be the same as before when the surfaces of the soil balls were examined. Mycorrhizas formed by *Hebeloma* sp. were seen on 14 of the 18 plants propagated in 'non-mycorrhizal' cores, while three bore an unidentified mycorrhizal type; in contrast, mycorrhizas formed by *Inocybe* sp. were seen on 16 of the 18 plants propagated in 'mycorrhizal' soil, while 4 had BCT mycorrhizas and 2 bore mycorrhizas of *Laccaria* sp. (Table 4.6.1).

One year later (June 1980), 17 of the 18 plants from 'non-mycorrhizal' cores bore mycorrhizas of *Hebeloma* sp. and 7 bore mycorrhizas attributable to *Lactarius pubescens*. In contrast, 14 of the 18 plants from 'mycorrhizal' cores had mycorrhizas attributable to *Inocybe* sp., while 13 had BCT; mycorrhizas attributable to *Hebeloma* sp. were uncommon in this treatment, being seen on only 3 and 2 plants respectively (Table 4.6.1).

Occurrence of fruitbodies before outplanting:

Fungal fruitbodies appeared above ground in many pots between 9 August and 8 November 1979 (Table 4.6.2); in 1980 they appeared again in many pots, beginning one month earlier (4 July). Fruitbodies of *Hebeloma* (possibly *H. crustuliniforme*) predominated around plants from 'non-mycorrhizal' cores, thus matching the occurrence of mycorrhizas attributable to *Hebeloma*; 9 such plants supported fruiting by *Hebeloma* in 1979, and 7 plants in 1980. Fruitbodies of *Thelephora terrestris* and *Peziza badia* Persoon ex Mérat were seen around one plant in each case in 1979 and around two plants in 1980. In contrast to these observations for 'non-mycorrhizal' plants, fruitbodies of *Inocybe lanuginella* predominated around plants from 'mycorrhizal' cores; such

TABLE 4.6.1: Effects of 'mycorrhizal' and 'non-mycorrhizal' propagating soils on the sequence of mycorrhizas found in succeeding years on roots of developing seedlings of *Betula pendula*.

Type of sheathing mycorrhiza (or fruitbody)	Numbers of seedlings (maximum shown in last row) on which different mycorrhizal types occurred									
	Non-mycorrhizal propagating soil					Mycorrhizal propagating soil				
	1979	1980	1981	1982	1979-1982	1979	1980	1981	1982	1979-1982
Brown with cream tips	0	0	1(0)*	3(0)	4	4(0)	13(0)	7(0)	5(0)	17
Hebeloma sp.	14(9)	17(7)	6(1)	4(2)	18	0	2(0)	3(0)	4(0)	9
Inocybe lanuginella	0	0	7(0)	5(0)	12	16(16)	15(12)	7(0)	5(0)	18
Lactarius pubescens	0	7(0)	6(0)	5(3)	15	0	3(0)	1(0)	2(1)	4
Thelephora terrestris	0(1)	4(2)	0	0	4	0	0	0	0	0
Peziza badia	0(1)	0(2)	0	0	2	0	0	0	0	0
Laccaria sp.	0	0	0	0	0	2(2)	2(2)	0	0	3
No. seedlings examined	18	18	7	5	18	18	18	7	5	18

* Numbers in parentheses indicate numbers of seedlings on which fruitbodies attributable to the fungus were produced.

TABLE 4.6.2: Dates in 1979 when newly formed fruitbodies of sheathing mycorrhizal fungi were found in pots containing seedlings of *Betula pendula* propagated earlier in the year in 'mycorrhizal' or 'non-mycorrhizal' soils.

	Numbers of pots containing different types of fruitbody*																			Total No. pots with fruitbodies
9 August	1I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
16 August	-	2I	3I	1I	1I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7
24 August	-	-	-	-	-	1I	-	-	-	-	-	-	-	-	-	-	-	-	-	1
29 August	-	-	-	-	-	-	1L	1I	1I	-	-	-	-	-	-	-	-	-	-	3
3 September	-	-	-	1I	-	-	-	-	-	1I	-	-	-	-	-	-	-	-	-	2
16 September	-	-	-	-	-	-	-	-	-	-	1P	1T	-	-	-	-	-	-	-	2
21 September	1I	-	-	-	1I	-	-	1I	-	-	-	-	1I	1H	-	-	-	-	-	5
1 October	-	2I	-	-	-	1I	-	-	1L	-	-	-	-	-	3I	1H	3H	-	-	11
19 October	-	-	-	-	-	-	1L	-	-	-	-	-	-	1H	-	1H	-	2H	-	5
8 November	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2H	2

* Entries in the same column indicate recurrence of fruitbody production in the pots.

L, *Laccaria* sp.; P, *Peziza badia*; H, *Hebeloma* sp.; T, *Thelephora terrestris*; I, *Inocybe lanuginella* fruitbodies were seen around 16 of 18 plants in 1979 and 12 of 18 plants in 1980, with fruitbodies of *Laccaria tortilis* ([Bolt] S.F. Gray) Cke. being seen on two plants in each year. These observations again match the occurrence of mycorrhizas below ground.

In a few instances fruitbodies appeared in pots where no corresponding mycorrhizal type was seen, probably because observations of mycorrhizal types were confined to the surfaces of the soil balls. However, fruitbodies of *P. badia* were never associated with a distinctive mycorrhizal type, perhaps because *P. badia* was not growing as a mycorrhizal symbiont. It is notable that fruitbodies of *L. pubescens* were never seen in the glasshouse, despite the presence of mycorrhizas attributable to *L. pubescens* in 10 of the 36 pots.

Fruitbodies seen in the glasshouse were of similar size to those seen in field conditions. Often only one fruitbody appeared in each pot, near the rim, but fruiting occurred twice in 11 pots in 1979. Interestingly, the fruitbodies appeared in flushes in 1979, the first (*I. lanuginella*) early in August and the second (*Hebeloma* sp., *Inocybe* sp. and *Laccaria* sp.) at the end of September and beginning of October (Table 4.6.2).

Mycorrhizas and fruiting after outplanting:

At the end of the third (27 January 1982) and fourth (18 November 1982) growing seasons, the major differences in mycorrhizal status established in the glasshouse were still apparent (Table 4.6.1). Thus, mycorrhizas attributable to *L. pubescens* were present on many plants propagated in 'non-mycorrhizal' cores, and in large numbers on those plants; mycorrhizas of *Hebeloma* sp. were also usually present, but in much lower numbers. In contrast, BCT mycorrhizas were predominant on plants propagated in 'mycorrhizal' soil, whereas mycorrhizas of

Hebeloma sp. or *L. pubescens* were uncommon in this treatment. Mycorrhizas of *Inocybe* sp. were present on the root systems irrespective of propagation treatment, but were never numerically dominant, accounting for 27% and 34% of total mycorrhizal tips of plants propagated in 'mycorrhizal' and 'non-mycorrhizal' cores respectively.

Few fruitbodies were seen in the field, despite regular observation; combining the results for the third and fourth growing seasons, fruitbodies of *L. pubescens* were seen in low numbers around 4 trees in the 'non-mycorrhizal' group and around only one tree in the 'mycorrhizal' group, and fruitbodies of *Hebeloma* (identifiable as *H. crustuliniforme* in one case) were seen around 3 'non-mycorrhizal' trees in 1982. These observations extended information on the occurrence of *L. pubescens*, which is known to have been present on 14 of 18 plants propagated in 'non-mycorrhizal' soil but on only 4 of 18 plants in the 'mycorrhizal' propagation treatment (Table 4.6.1).

The spatial distributions of different types of mycorrhiza are summarised for the third and fourth growing seasons in Figure 4.6.1 where the first two distance intervals (0-6 and 10-11 cm) can be taken to represent the part of the root system present before outplanting. The most numerous type of mycorrhiza close to the tree base was attributable to *L. pubescens* on trees propagated in 'non-mycorrhizal' soil but to BCT on trees from 'mycorrhizal' soil. Evidently, the fungi responsible for these mycorrhizas had spread on the roots as these grew out into the field soil, but at greater distances from the tree base the roots, irrespective of propagation treatment, had been colonised by *Inocybe*. Figure 4.6.1 shows also that the proportion of mycorrhizas attributable to *Hebeloma* was consistently greater on all parts of the root systems of trees propagated in 'non-mycorrhizal' than in 'mycorrhizal' soil.

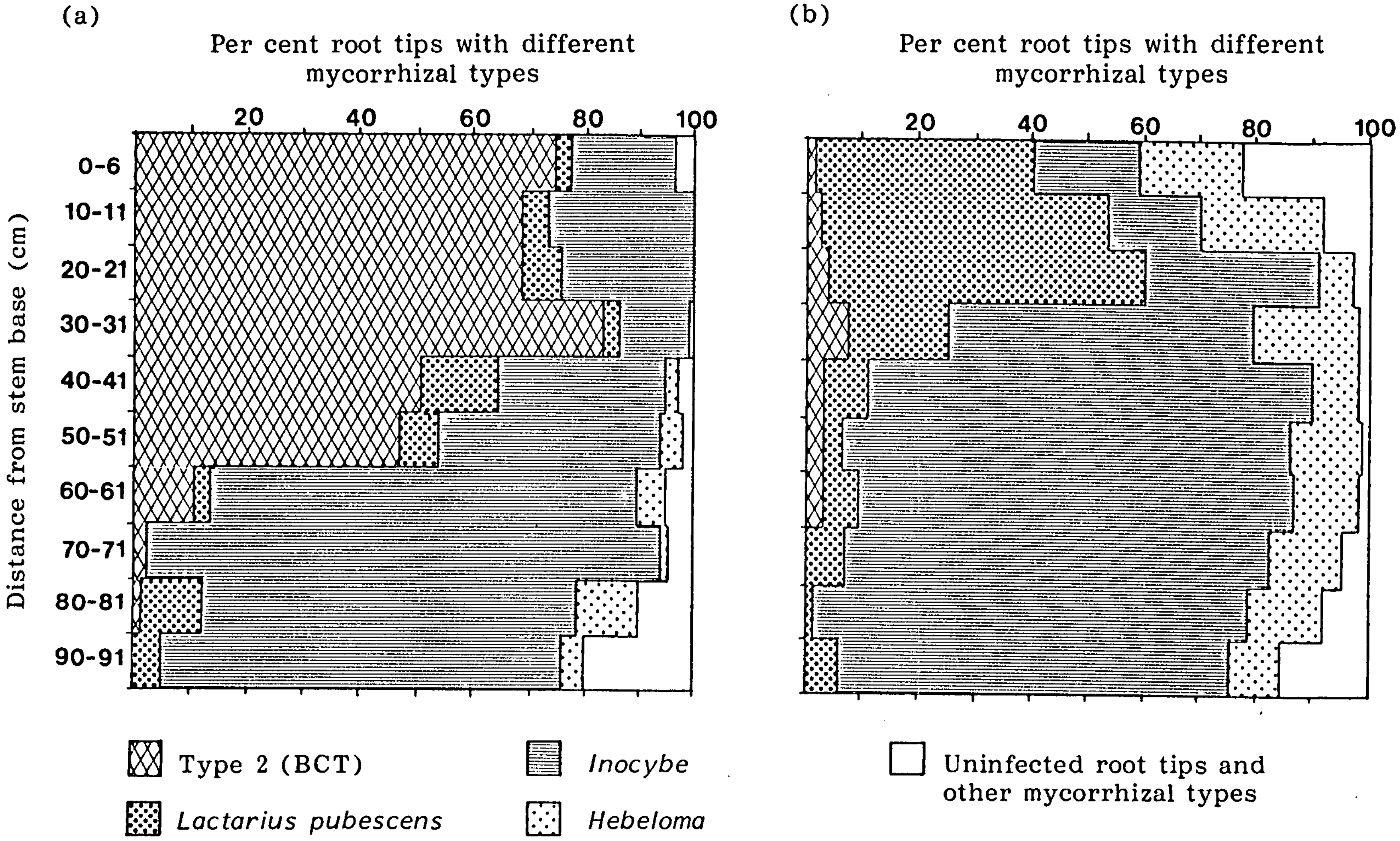

FIGURE 4.6.1: Frequency of occurrence of different types of mycorrhiza at distances from the stem bases of birch seedlings propagated initially in (a) 'mycorrhizal' soil and (b) 'non-mycorrhizal' soil and transplanted to a field site; combined data from assessments of 12 replicate trees when 35 and 45 months old.

In order to determine the likely sources of inocula for the outgrowing roots, ten soil cores were taken from gaps in the outplanting site on 25 February 1982 and sown to birch in a glasshouse. The seedlings grown for 15 weeks bore a predominance of Hebeloma-type mycorrhizas and some attributable to *Inocybe*; neither *Lactarius* nor the fungus responsible for BCT formed mycorrhizas on the seedling roots. It thus seems that *Inocybe* sp. and *Hebeloma* sp. could have colonised the outgrowing roots of field-grown plants from either soil inocula or pre-existing infections, whereas *L. pubescens* and the fungus forming BCT are likely to have spread from established infections.

In summary, this experiment demonstrates three major points.

1. There is a changing pattern of colonisation of roots with time; this is seen most clearly on the plants propagated in 'non-mycorrhizal' soil. The early dominance of *Hebeloma* sp. was replaced eventually by dual dependence of the plants on mycorrhizas formed by *L. pubescens* and *I. lanuginella*, though not to the complete exclusion of *Hebeloma* sp.

2. There is a systematic distribution of mycorrhizas of different types in space. Thus, BCT mycorrhizas or those attributable to *L. pubescens* occupied the largest proportion of roots near the tree base, whereas mycorrhizas formed by *Inocybe* sp. were predominant in the younger parts of the root system. It should, nevertheless, be stressed that for the root system as a whole *Inocybe* sp. was not the dominant fungus because it was present mainly in the part of the root system where there were fewest root tips.

3. There was a profound effect of the 8-week propagation period on the subsequent mycorrhizal development of seedlings during the next 45 months.

4.7 Discussion

The experiments in this section have shown clear differences between mycorrhizal fungi in their abilities to establish mycorrhizas on seedlings in unsterile soil and also in their abilities to persist, and subsequently to spread along root systems, when seedlings are transplanted into soils. In many respects, these features were related: the 'late-stage' fungi like *Amanita muscaria* and *Leccinum* spp. which could not establish on seedlings from soil inocula (Deacon *et al.*, 1983; Section 4.2) also did not persist when seedlings on which they were artificially established were transplanted into soil (Tables 4.5.3 and 4.5.5). Conversely, the 'early-stage' fungi, like *Hebeloma sacchariolens* and *Thelephora terrestris* which readily established mycorrhizas on seedlings in unsterile soil (Tables 4.3.5 and 4.3.13), also persisted on the root systems when established in pure culture and transplanted into soil (Tables 4.3.5 and 4.3.13). It therefore seems that categories 'early-stage' and 'late-stage' can be extended to apply not only to establishment on seedlings but also to subsequent persistence and spread on the root systems. But this extension of the concept must be provisional at this stage, because only few fungi have been adequately tested to date and even in these cases there were some anomalous results especially concerning *Paxillus involutus* which is considered later in the discussion.

In terms of establishment of a mycorrhizal symbiosis, the results in Section 4.2.1 showed clearly that *A. muscaria* and *L. scabrum* do not initiate infections of previously uninfected roots or of those previously infected by 'early-stage' mycorrhizal fungi in unsterile soil. There is thus no evidence of a 'priming' effect of 'early-stage' fungi in enabling 'late-stage' mycorrhizal fungi to develop. But the results for *Lactarius pubescens* in this experiment were difficult to interpret. *L. pubescens* is a 'late-stage' fungus by the criterion of Deacon *et al.* (1983) insofar as it cannot readily infect seedling roots from isolated sources of inoculum in soil, and this was confirmed by its inability to establish mycorrhizas from inoculum added to soil (Section 4.2.1). Yet this fungus did eventually develop on seedling roots, in the second growing season especially, and it did so as well in the absence of added inoculum as in its presence, presumably because it developed from resident sources of inoculum in the soil or from air-borne spores. Precisely the same phenomenon was observed in the experiment in Section 4.6, and it is tempting to suggest that this fungus is particularly active in establishing mycorrhizas after the host has gone through a dormant phase. The experiment in Section 4.4. again supports the view that *L. pubescens* is most active in colonising roots after they have been growing in soil for some time. When the fungus was established artificially on seedling root systems in pure culture and the seedlings were transplanted to soil, *L. pubescens* initially showed evidence of decline but it subsequently recovered such that it was present at a higher level after 147 days than after 49 days' incubation of the seedlings. In all these respects, *L. pubescens* seemingly has a different mode of behaviour from that of *A. muscaria* and *Leccinum* sp. because it develops relatively early on seedling root systems. It is not clear if the *Cortinarius* sp. investigated in Section 4.2.2 is a 'late-stage' fungus or not because it was not possible to be certain if type 5 mycorrhizas could be attributed to this fungus. Furthermore, because the fungus could not be grown pure culture syntheses of its mycorrhizas on birch seedlings could not be done and likewise neither could artificial inoculum be added to soil.

In terms of persistence, the experiments in Sections 4.5.1 and 4.5.2 showed clearly that the 'late-stage' fungi *A. muscaria* and *L. scabrum* are unable to maintain themselves on seedling root systems when artificially established in pure culture and then introduced into unsterile soil. This has been reported previously by Mason *et al.* (1983a) for a different isolate of *A. muscaria* and it is true even if the soil had previously been steam-sterilised (Section 4.5.1). The reasons for the failure to persist are unknown and unfortunately an experiment designed to see if interactions with other soil micro-organisms were responsible by using a sterile rooting medium was unsuccessful (Section 4.5.3). Nevertheless, the results show that it is pointless to try to establish such 'late-stage' fungi on seedling roots, even if this could be achieved in practice, because the fungus would not persist when the plants are introduced into unsterile soil. Again, *L. pubescens*, although a 'late-stage' fungus (*sensu* Deacon *et al.*, 1983), seemed to differ from *A. muscaria* and *L. scabrum* in this respect (Section 4.4): it did persist, albeit only poorly at first, on roots of seedlings planted into unsterile soil.

The 'early-stage' fungi *H. sacchariolens*, *T. terrestris* and *Laccaria proxima* showed a completely different pattern of behaviour from that of the 'late-stage' fungi. They established readily on seedling roots from inoculum sources in soil (Sections 4.3.1, 4.3.2 and 4.5.2) and they persisted and spread on the root systems after primary inoculation of the seedlings (Sections 4.2.1, 4.3.1 and 4.3.2). They did, however, show interesting interactions, one with another, in these respects and in some cases they showed also different patterns of behaviour in different soil environments. Thus, in brown earth both *T. terrestris* and *H. sacchariolens* persisted on roots on which they were initially established, often to the exclusion of resident or 'contaminant' mycorrhizal types which developed on uninoculated seedling roots transplanted to unsterile soil. But of these two fungi, *H. sacchariolens* was the most 'competitive' because it could colonise outgrowing roots of seedlings on which *T. terrestris* was established (Sections 4.3.1 and 4.3.2), whereas *T. terrestris* had less effect on pre-established infections by *H. sacchariolens*. In peat, however, the status of these two fungi was reversed: *H. sacchariolens* persisted relatively poorly on pre-colonised root systems and it did not exclude subsequent colonisation by *T. terrestris* (Section 4.3.2); also, in peat *H. sacchariolens* did not colonise roots from soil-applied inoculum whereas *T. terrestris* did so. Mason *et al.* (1983a) have also reported the failure of *H. sacchariolens* to persist on birch seedlings planted out into peaty soils - the fungus maintained itself in the first growing season but then completely disappeared in the second growing season, whereas it persisted well in seedlings planted into mineral soils.

The change in mycorrhizal status from one season to the next, reported for *H. sacchariolens* by Mason *et al.* (1983a) and seen also in the case of *L. pubescens* (Sections 4.2.1 and 4.6) is particularly interesting. It is consistent with the view expressed by Marks and Foster (1967) that an interruption to root growth, such as may occur with host dormancy and the initiation of a new season's root growth, may be a critical factor enabling the replacement of one mycorrhizal symbiont by another. In this respect, the results from my studies must be interpreted with caution because few of the experiments on replacement involved a period of dormancy. Yet, in the event, the use of glasshouse conditions in my experiments may have paralleled this situation: roots are likely to grow more rapidly in glasshouse than in field conditions, so the glasshouse provides a critical environment in which to test the ability of a pre-established fungus to 'keep pace' with growing roots - equivalent in some respects to the ability of a fungus to grow rapidly enough (Bowen and Theodorou, 1973) to colonise roots as they initiate regrowth (Wilcox, 1968) after a period of host dormancy, otherwise the roots may be colonised by fungal propagules in the soil (Theodorou, 1980).

The behaviour of *P. involutus* in my experiments merits special comment. This fungus has not formally been placed into either the 'early-stage' or the 'late-stage' category in the sense of Deacon *et al.* (1983), mainly because it is not a typical colonist of birch roots in mineral soils which were used by Deacon *et al.* (1983). Rather, *P. involutus* most commonly occurs as a mycorrhizal symbiont in coal spoil and other reclamation sites around Edinburgh and one of the isolates used in this study was derived from colliery spoil. F.M. Fox (personal communication) has shown that *P. involutus* will establish mycorrhizas on birch roots from basidiospores applied to coal spoil but it does so only poorly from basidiospores added to brown earth (Fox, 1983), similarly excised mycorrhizal roots were shown earlier to behave as inoculum in exactly the same manner (Section 3.3.1). It could thus be considered as either an 'early-stage' or 'late-stage' fungus with a clear soil-dependency in its behaviour. In my experiments, one isolate of *P. involutus* did not maintain itself on seedlings in unsterile brown earth and it did not colonise them from soil inocula. But a second isolate behaved peculiarly - it persisted only poorly on pre-colonised root systems when the plants were transferred to either brown earth or peat, yet in both rooting media it colonised the outgrowing roots extensively from soil-borne inoculum, even if the plants initially bore mycorrhizas of other 'early-stage' fungi. This behaviour cannot be explained except by the assumption that this isolate of *P. involutus* relies more on recurrent infections from soil-borne inoculum than on ectotrophic growth from pre-established infections on the roots. If so, then this fungus more closely resembles saprophytic rhizosphere fungi (Taylor and Parkinson, 1961) than other mycorrhizal fungi (Robertson, 1954). This is inconsistent with the report that isolate 16 spread well on the roots of birch (Mason *et al.*, 1983a) and isolate 32 spread with the roots of Sitka spruce (P.A. Mason, personal communication) when transplanted to field soils. *P. involutus*, like *L. pubescens*, would seem to merit much more detailed study than it has received to date, because its behaviour (a) seems to fall between that of 'typical' 'early-stage' and 'late-stage' mycorrhizal fungi and (b) is seemingly markedly affected by soil conditions, enabling critical experiments to be done in an attempt to understand the host-parasite interaction.

The final experiment reported here (Section 4.6) is interesting because it demonstrates clearly spatial and temporal changes in mycorrhizal status of trees propagated in two 'soils'; the experiment thus supports and confirms the work of Ford *et al.* (1980), Mason *et al.* (1982) and Deacon *et al.* (1983). Thus, the different types of sheathing mycorrhizas are not randomly arranged but show recognisable distributions. Yet, the most interesting and potentially important result from this work relates to the considerable influence of events during the 8-week period of propagation on the development of mycorrhizas during the next 45 months when the seedlings were treated alike in all respects. Plants propagated in 'mycorrhizal' soil from beneath the canopy of a tree developed type 2 ('brown with cream tips') mycorrhizas or mycorrhizas attributable to *Inocybe* sp. and these types remained dominant throughout the 4 years of the experiment. In contrast, plants propagated in the same soil but without a history of tree growth developed mycorrhizas of *Hebeloma* sp., succeeded by a mixture of *Inocybe* sp. and *L. pubescens* though never to the complete exclusion of *Hebeloma* sp. These and other results in this section (4) demonstrate that it is possible, in practice, to influence the types of mycorrhiza that develop on the roots of birch after outplanting, by appropriate manipulations during propagation. In doing so, however, it must be recognised that other factors such as soil type and rhizosphere micro-organisms may significantly affect the successional patterns, and little is yet known about the influence of seasonal cycles of root dormancy and regrowth on the replacement of one mycorrhizal fungus by another.

SECTION V

Establishment of Mycorrhizas on Birch Seedlings Planted Around Mature Trees

5.1 Introduction

As explained in Section 1.5.1, Deacon *et al*. (1983) distinguished two types of mycorrhizal fungus, termed 'early-stage' and 'late-stage', on the basis that birch seedlings grown in soil cores taken from beneath the fruitbodies of late-stage fungi did not become infected by these fungi, unlike the case with 'early-stage' fungi. Similarly, when vermiculite-peat inocula of 'late-stage' fungi such as *Lactarius pubescens* and *Leccinum* spp. were added to soil there was little or no infection of seedling roots by these fungi (Deacon *et al*., 1983; see also Section 4.2.1).

A common, though not exclusive, feature of the reported late-stage fungi, and especially of *L. pubescens* and *Leccinum* spp., is that they form conspicuous mycelial strands. Most work on such mycelial strands has concentrated on their ability to take up and translocate to the host substances such as phosphate and water from the soil environment (e.g. Skinner and Bowen, 1974a; Duddridge *et al*., 1980). But Garrett (1951) considered that the primary function of mycelial strands in pathogenic root-infecting fungi is as agents of infection. The role of mycelial strands in initiating infection by mycorrhizal fungi has been recognised by a number of workers (Young, 1937; Robertson, 1954; Ashton, 1976) and was recently emphasised by Chilvers and Gust (1982). Yet their postulated roles in the infection process have not been fully investigated, so the experiments described here and later were intended to assess their significance for mycorrhizal establishment.

In the case of some pathogenic fungi, notably *Armillaria mellea* (Vahl. ex Fr.) Kummer, the infectivity of mycelial strands or rhizomorphs depends on nutrients supplied by a food base (Garrett, 1956). It was thought possible that the failure of 'late-stage' mycorrhizal fungi to infect seedlings in soil cores could be due to the severance of the inoculum in the soil cores from the food base provided by the parent tree. This section describes experiments designed to investigate this possibility - experiments in which inoculum of mycorrhizal fungi was separated from contact with a parent tree by means of coring or trenching. Most of the experiments were done on an experimental birch plot at the Bush Estate, Penicuik, Midlothian. This plot of sixty trees was established from seed of twelve collections of *Betula pendula* and nine of *B. pubescens*. After a period of growth in a glasshouse, the seedlings were transplanted to the field, 3 m apart, in 1971. The site into which the seedlings were planted is adjacent to the I.T.E. on the Bush Estate, at an altitude of 200 m and with a mean annual rainfall of 800 mm. The soil is an imperfectly drained gley derived from clay-loam till and had borne only potatoes and soft fruit in the previous 30 years. Since 1975, fruitbodies of mycorrhizal, or suspected mycorrhizal, fungi have been recorded on a permanently marked grid (Mason *et al.*, 1982), and work has also been done on the distributions of mycorrhizas in the plot (Deacon *et al.*, 1983). Consequently, the 'history' of individual trees was available to facilitate further investigation.

In addition to the work done in the experimental birch stand, a limited study of the mycorrhizal colonisation of birch seedlings in soil from two contrasting woodland sites was made.

5.2 The Effect of Coring of Soil on the Ability of Two Mycorrhizal Fungi to Colonise Seedlings Grown Around Mature Trees

In order to investigate whether isolation from a mature tree affects the ability of 'late-stage' fungi (*sensu* Deacon *et al.*, 1983) to colonise seedlings in soil, trees with known distributions of such fungi were selected for study, in two experiments as follows.

5.2.1 *Tree N2, Bearing Fruitbodies of Lactarius pubescens*[1]

A 10-year-old tree of *Betula pubescens* (N2) was selected in the experimental birch plot at the Bush Estate. The distribution of mycorrhizas and fruitbodies around the tree had been recorded in previous years and the tree was known to have mycorrhizas of *L. pubescens* more or less evenly distributed around it to a distance of at least 1 m from the trunk. The ground beneath the tree was covered in black polyethylene sheeting from December 1980 until May 1981, to suppress growth by the herbaceous ground cover. Meanwhile, non-mycorrhizal seedlings of *B. pendula* were prepared by the method described in Section 2.3.2. The seed was sown into propagators on 27 March 1981 and the plants were ready for transplanting, after suitable hardening off, 55 days later. The shoots were then 20 ± 1.7 (s.e.) mm tall, weighed 3.6 ± 0.51 mg (oven-dry) and had a mean of 49 ± 5.3 uninfected root tips.

Forty-eight planting positions were chosen in four annuli around the tree, at 20, 35, 50 and 75 cm radii from the trunk (Figure 5.2.1). The planting positions were evenly spaced within the annuli so there were progressively more seedlings further out from the tree. Alternate positions were untreated and received one seedling each - these are referred to as 'non-isolated' seedlings. At other positions a core of soil (10 cm diameter, 8 cm depth) was taken with a corer on 2 June 1981 and the intact core was then replaced immediately with minimal disturbance; the roots within the core were thus severed from the parent tree. All plantings were done on 3 June 1981 by removing a narrow cylinder of soil with a cork borer (1 cm diameter) and inserting into the hole a root

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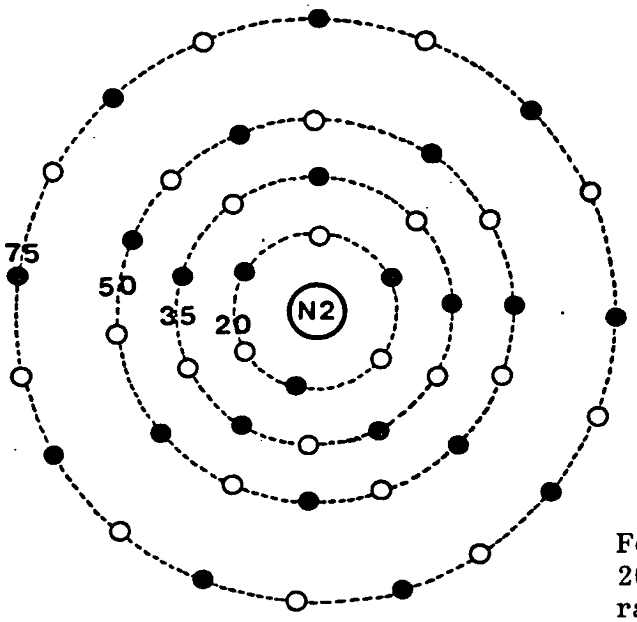
[1] This section (5.2.1) forms part of a paper, as follows: Fleming, L.V. (1983). Mycorrhizal succession on birch: infection of seedlings planted around mature trees. Plant & Soil 71: 263-267.

(a)



Four annuli at
20, 35, 50 and 75 cm
radii

(b)

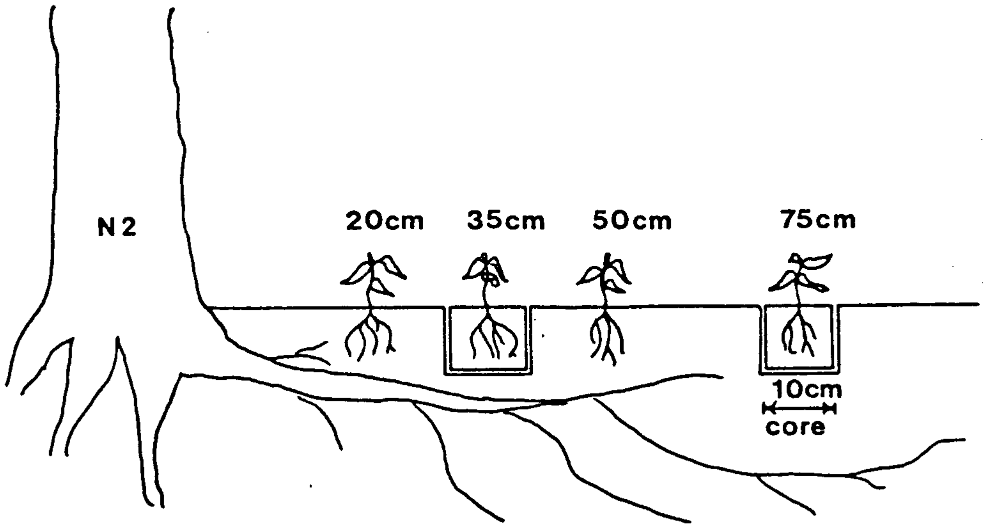


FIGURE 5.2.1: (a) Distribution of isolated (cored, ●) and non-isolated (o) birch seedlings around a 10-year old birch tree (N2) on the Bush Estate.

(b) Diagram to illustrate the occurrence of seedlings in cored and non-isolated positions.

system of a 55-day-old non-mycorrhizal seedling grown in a 1 cm diameter plastic sleeve (Section 2.3.2), the sleeve being removed at planting.

The seedlings were watered as required in the summer of 1981 and a 2 cm deep mulch of sphagnum peat was applied to the site on 3 August to help conserve moisture; total rainfall during the period of the experiment was 315 mm. At intervals, the cores containing the isolated seedlings were carefully lifted and replaced immediately to prevent re-invasion by roots or mycelial strands. Light intensities at seedling level were recorded using a 'Lambda' quantum meter at a number of fixed points within the experimental area throughout two cloudless days in August. Light intensities were very variable around the tree because of the aspect of the stand and the thin canopy. At 0930 hours the seedlings were receiving 62-100% of full daylight; 3 hours later the range of recorded light intensities had dropped to 18-64% of full daylight. Light intensities continued to decline throughout the afternoon until by 1830 hours no part of the recorded area was receiving more than 7% of full daylight. The positions of fungal fruitbodies were mapped on a permanent grid system as they appeared.

Seedlings were sampled 17 weeks after they had been planted into the field, when the shoots of the isolated seedlings were both taller (39 ± 1.4 mm) and heavier (37.6 ± 3.93 mg oven-dry) than of the non-isolated ones (34 ± 1.4 mm and 27.5 ± 3.53 mg). The seedling roots were washed and assessed for mycorrhizal types (Table 5.2.1).

There were no differences in total number of mycorrhizal root tips or total number of all root tips between the treatments; only a small proportion of the root tips in either treatment were uninfected (Table 5.2.1). Four predominant mycorrhizal types were seen on the seedling

TABLE 5.2.1: Occurrence of mycorrhizal types on birch seedlings grown for 17 weeks in isolated (cored) and non-isolated positions around a mature birch tree (N2).

	No. seedlings bearing each mycorrhizal type (No. with >20% root tips of that type in parentheses)		Mean No. root tips of each mycorrhizal type (means of log(x+1) in parentheses)		
Mycorrhizal type	Isolated (max. 24)	Non-isolated (max. 23)	Isolated	Non-isolated	LSD (p=0.05)
Hebeloma	6 *(3)***	19 *(4)*	16 *(0.346)*	18 *(0.894)*	*(0.394)*
Lactarius	3 *(2)****	22 *(17)*	3 *(0.145)*	64 *(1.663)*	*(0.318)*
Type 4	17 *(16)*	11 *(11)*	70 *(1.330)*	34 *(0.869)*	*(0.504)*
Type 5	19 *(13)*	17 *(9)*	54 *(1.279)*	47 *(1.118)*	*(0.488)*
Total No. mycorrhizal root tips/seedling			143 *(2.115)*	166 *(2.198)*	*(0.120)*
Total No. uninfected root tips/seedling			11 *(1.026)*	7 *(0.793)*	*(0.140)*
Total No. root tips/seedling			154 *(2.157)*	173 *(2.218)*	*(0.095)*

, difference significant at p=0.01; *, difference significant at p=0.001.

root systems. Unidentified types '4' and '5' were found on similar numbers of isolated and non-isolated seedlings and the numbers on these seedlings did not differ significantly (Table 5.2.1). By contrast Lactarius-type mycorrhizas were present on significantly more non-isolated than isolated seedlings and they were frequently the dominant type on non-isolated seedlings (in 11 out of 23 instances) but were never dominant on isolated seedlings. The difference in the case of Lactarius-type mycorrhizas can be illustrated in another way: a total of 73 mycorrhizal tips of this type was recorded on the 24 isolated seedlings, compared with 1469 on the 23 non-isolated seedlings (one of the original 24 seedlings in this category died). There was thus a twenty-fold difference in the numbers of Lactarius-type mycorrhizas between the two treatments.

Hebeloma-type mycorrhizas were present on many non-isolated seedlings but not on the isolated seedlings. In most cases, however, they were present in very low numbers and if the value of 20% of total root tips represented by Hebeloma-type mycorrhizas is taken as an arbitrary indication of good establishment then Table 5.2.1 shows that few seedlings in either category were heavily colonised by it. In fact, 389 mycorrhizal tips of *Hebeloma* sp. were recorded on the 24 isolated seedlings, compared with 408 on the 23 non-isolated seedlings.

The distribution of mycorrhizal types on the parent tree root system was examined early in November 1981 by taking soil cores (8 cm deep and 2 cm diameter) at 25 cm distance intervals, out to 1.25 m, on ten radiating transects from the tree trunk. Lactarius-type mycorrhizas represented only 12.0% of the total number of mycorrhizal root tips, the highest recorded value being 31.0% for the ten cores at 100 cm distance from the trunk (Table 5.2.2). Type '5' mycorrhizas were the

TABLE 5.2.2: Mean percentage of mycorrhizas attributable to different types in sets of ten soil cores collected at five distances from a mature birch tree (N2) and on seedlings grown in 'isolated' and 'non-isolated' positions around the tree in the field.

	Mycorrhizal type				
Distance (cm)	Hebeloma	Lactarius	Type '4'	Type '5'	Others
25	14.9	4.6	8.7	71.8	
50	30.9	3.1	10.9	55.0	
75	14.2	21.3	9.1	40.2	15.1
100	30.5	31.5	12.7	25.4	
125	58.9	11.9	9.3	19.9	
Total	26.1	12.0	9.9	48.9	3.2
'Isolated' seedlings (in cores)	11.2	2.1	48.9	37.8	
'Non-isolated' seedlings	10.8	38.5	20.5	28.3	

dominant type, occupying 48.8% of root tips overall; Hebeloma-type accounted for 26.1%, type '4' mycorrhizas for 9.9% and other types for 3.2%. Thus, comparing the results for seedling and parent tree root systems, only Lactarius-type mycorrhizas were markedly over-represented on the non-isolated seedlings and under-represented on the isolated seedlings; *Hebeloma* sp. and type '5' were under-represented on the seedling roots in general, and type '4' was over-represented on the seedling roots in general.

Most fruitbodies that appeared around the tree in autumn 1981 were of *L. pubescens*, *Hebeloma velutipes* Bruchet and a *Cortinarius* sp. (Figure 5.2.2). Three species of *Russula* were also observed (*R. versicolor* J.Sch., *R. betularum* Hora and *R. grisea* (Secr.) Gill.), but neither they nor the *Cortinarius* sp. seemed to represent the unidentified mycorrhizal types on the roots. Interestingly, both Hebeloma-type and Lactarius-type mycorrhizas were present in their highest proportions in the soil cores taken at the distance at which their fruitbodies were most common (c.f. Table 5.2.2 and Figure 5.2.2).

L. pubescens was the only mycorrhizal fungus of those observed that formed mycelial strands; indeed, it was the only late-stage fungus (*sensu* Deacon *et al.*, 1983) that occurred on the seedlings, *Russula* spp. and *Cortinarius* sp. being excluded from consideration because they apparently did not infect seedling roots.

The results show clearly that *L. pubescens* can establish mycorrhizas on seedlings in soil, provided that the fungus is not separated from the parent tree; furthermore, it can colonise seedling roots to a greater degree than its level of establishment on the parent tree root system would suggest. This is consistent with the view that *L. pubescens* colonised seedling roots by means of mycelial strands or,

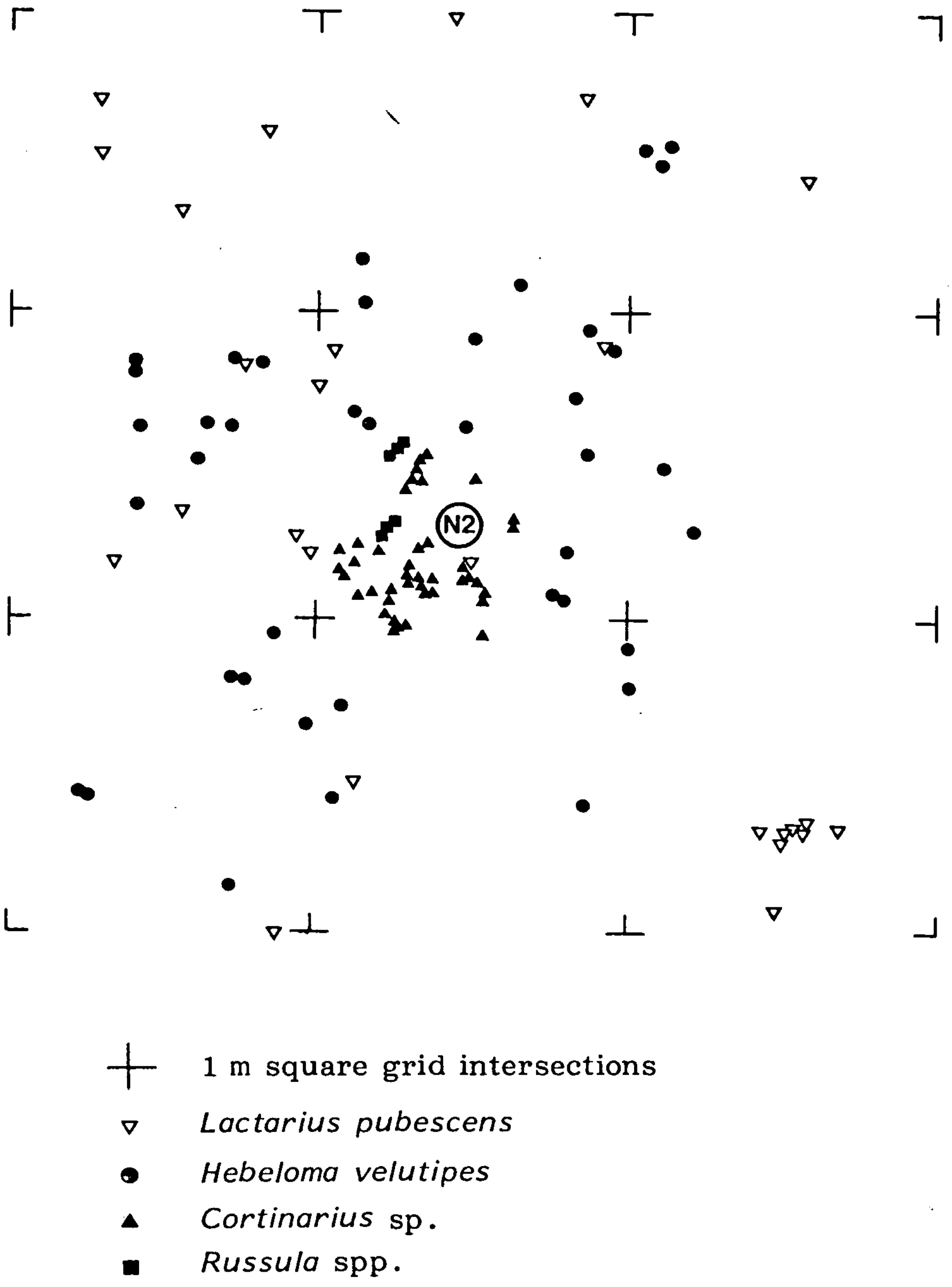


FIGURE 5.2.2: Distribution of fruitbodies of mycorrhizal, or suspected mycorrhizal fungi around a 10-year old birch tree (N2) in autumn 1981.

at least, by hyphae that needed to remain attached to the food base provided by the parent tree. In this respect, the results parallel those previously obtained for root-infecting pathogens such as *Armillaria mellea* (Garrett, 1956).

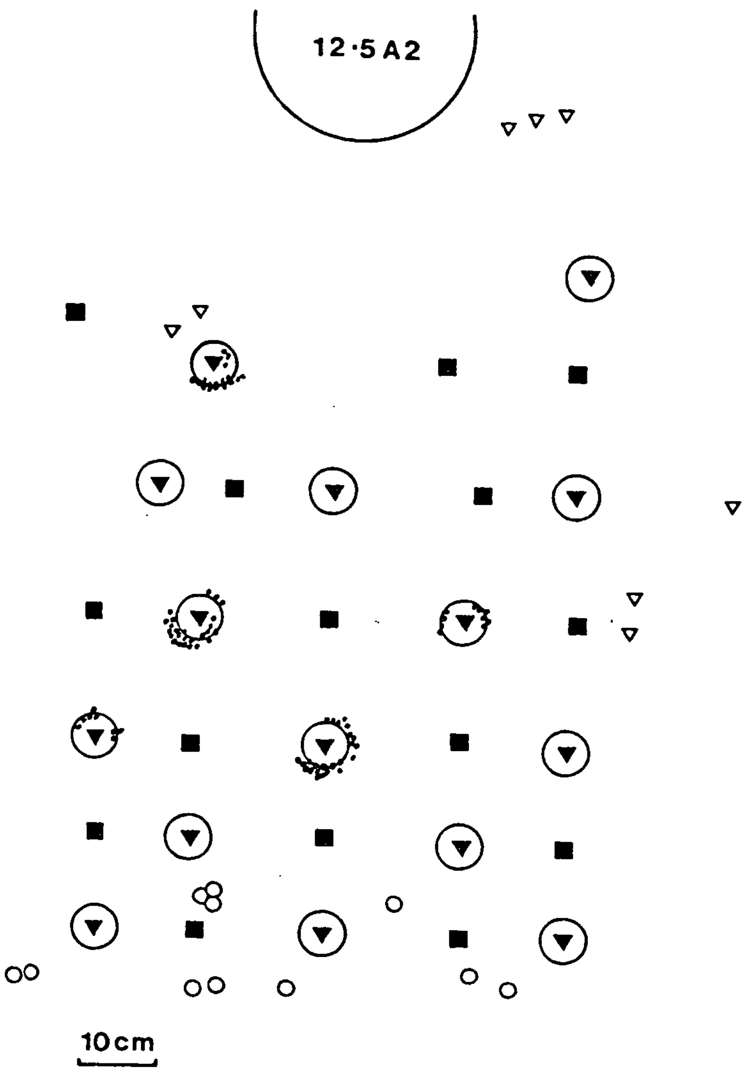
The coring technique used in this experiment seems to have been sufficient both to maintain continued isolation of the inoculum in the soil core from the parent tree and to allow adequate seedling growth in the core for at least one growing season. Indeed, seedlings grown in the cores in the field were larger than those grown in the non-isolated positions; this is possibly because the isolated seedlings did not have to compete with the parent tree for water or mineral nutrients, but the possible involvement of different mycorrhizal fungi in the differential growth of seedlings cannot be excluded.

5.2.2 Tree 12·5A2, Bearing Fruitbodies of Tricholoma fulvum

It was demonstrated that *Lactarius pubescens* can infect seedlings in soil provided that the fungus is not separated from the food base provided by a mature tree (Section 5.2.1). *Tricholoma fulvum* (DC. ex Fr.) Sacc. has some similarities to *L. pubescens* in that the mycorrhizas of *T. fulvum* produce mycelial strands and the fruitbodies of this fungus have not been observed around young birch saplings but only after several years' growth in the field. It was therefore of interest to see if *T. fulvum* exhibits similar behaviour to that of *L. pubescens* with regard to the colonisation of seedlings in soil.

Fruitbodies of *T. fulvum* were observed in autumn 1981 around an 8-year-old tree of *B. pendula* (termed 12.5A2) growing on the Bush Estate, Penicuik, Midlothian. The positions of these fruitbodies were noted and the ground beneath the tree was mulched with black polyethylene sheeting over the winter of 1981/82. Thirty planting positions were chosen in the area where the fruitbodies of *T. fulvum* had been observed (Figure 5.2.3). The positions were arranged in six rows of five, spaced 20 cm apart, so the area covered by the experiment formed a block starting at 30 cm distance from the tree and extending to 130 cm distance. Alternate planting positions were cored on 2 June 1982 and the cores were carefully replaced (Section 5.2.1). A 19-week-old non-mycorrhizal birch seedling, prepared by the method described in Section 2.3.2, was then planted into each position on 3 June 1982; at planting the seedlings bore 113 ± 20.0 (s.e.) uninfected root tips and had shoots that were 36 ± 13.5 mm tall and weighed 8.3 ± 4.17 mg (oven-dry). The cores were lifted at intervals, a peat mulch was applied, and the seedlings were watered when necessary, just as in the previous experiment.

The seedlings were harvested 14 weeks later, on 8 September 1982. There was then no significant difference between the treatments in either shoot height (isolated seedlings 54 ± 5.3 mm; non-isolated 50 ± 3.6 mm) or shoot oven-dry weight (isolated seedlings 86.4 ± 21.94 mg; non-isolated 49.3 ± 9.41 mg). There was also no significant difference between the treatments in either the total number of mycorrhizas or the total number of root tips (Table 5.2.3). Mycorrhizas of *Hebeloma* sp., *Laccaria* sp. and *Thelephora terrestris* were the predominant types on the seedling root systems; there was, however, no significant difference between the treatments in the numbers of seedlings infected by these types nor in the mean number of mycorrhizal tips of these types observed on the seedlings. No Tricholoma-type mycorrhizas were observed on the roots of the isolated seedlings, and only four non-isolated seedlings had any mycorrhizas of this type, these being few in number; this difference was not significant (Table 5.2.3).



◯ Position of soil cores (10 cm diameter)

○ Fruitbodies of *Hebeloma* sp.

▽ " *Tricholoma fulvum*

• " *Laccaria torilis*

FIGURE 5.2.3: Distributions of isolated (▼) and non-isolated (■) seedlings in relation to the fruitbodies of mycorrhizal fungi around a mature birch tree (12-5 A2).

TABLE 5.2.3: Occurrence of mycorrhizal types on birch seedlings grown for 14 weeks in isolated (cored) and non-isolated positions around a mature birch tree (12.5 A2).

	No. seedlings bearing each mycorrhizal type		Mean No. root tips of each mycorrhizal type (means of log(x+1) in parentheses)		
Mycorrhizal type	Isolated (max. 12)	Non-isolated (max. 14)	Isolated (n=12)	Non-isolated (n=14)	LSD (p=0.05)
Tricholoma	0	4	0 *(0.000)*	18 *(0.504)*	*(0.664)*
Hebeloma	5	5	46 *(0.754)*	27 *(0.645)*	*(0.779)*
Thelephora	5	2	33 *(0.731)*	23 *(0.316)*	*(0.705)*
Laccaria	7	10	84 *(1.253)*	55 *(1.251)*	*(0.807)*
Cenococcum	1	1	3 *(0.131)*	5 *(0.131)*	*(0.383)*
Others	1	5	1 *(0.095)*	14 *(0.566)*	*(0.506)*
Uninfected	11	9	13 *(0.920)*	3 *(0.469)*	*(0.358)*
Total number of mycorrhizal root tips			167 *(2.000)*	141 *(2.033)*	*(0.582)*
Total number of root tips			179 *(2.227)*	144 *(2.040)*	*(0.253)*

Within the experimental area, seventy-three fruitbodies of *Laccaria tortilis* were recorded, this being the most numerous fruitbody type. *L. tortilis* was the only fungus that fruited on the cores themselves. Eleven fruitbodies of *Hebeloma* sp. were also recorded in the experimental area, but only five fruitbodies of *T. fulvum* were observed and these occurred just within the experimental area; an adjacent area, however, produced over twenty fruitbodies of this fungus. It was therefore thought possible that the failure of *T. fulvum* to infect the seedlings could have been due to a lack of inoculum in the area where the seedlings were planted. Accordingly, fifteen soil cores (8 cm deep and 2 cm diameter) were collected at random from within the planted area in January 1983 and examined for mycorrhizas present in them. Tricholoma-type mycorrhizas were present in nine of the fifteen cores and accounted for 64.3% of the 2633 mycorrhizal tips examined. Only two other mycorrhizal types were found in the small soil cores taken at this stage; they were Laccaria-type and Hebeloma-type and they represented 25.0% and 10.6% respectively of the mycorrhizal tips. There is thus no obvious reason why *T. fulvum* failed to develop - at least on the non-isolated seedling roots - unless competition from *Laccaria* sp., *Hebeloma* sp. and other 'early-stage' fungi excluded *T. fulvum.*

5.3 The Effect of Trenching Large Root Volumes on the Ability of Some Mycorrhizal Fungi to Infect Birch Seedlings

Lactarius pubescens was shown to infect seedlings in soil if its inoculum was not severed from the food base provided by a mature tree, whereas seedlings grown in soil cores around the tree were not colonised by this fungus (Section 5.2.1). With fungi such as *Armillaria mellea* both the growth rates of rhizomorphs and their 'vigour of infection' increase with the size of the inoculum (Garrett, 1956). In the case of *L. pubescens* it could thus be argued that failure to infect seedlings in soil cores could be because the small volume of the core did not contain sufficient mycelium or mycelial nutrient reserves to initiate infection. Alternatively, *L. pubescens* may rely on a direct and continuing supply of host-derived assimilates in order to be able to colonise seedling roots. The following two experiments were done to investigate the possibility that a large root volume, isolated from a supply of photosynthate, may provide a food base for infection by *L. pubescens* and other 'late-stage' fungi.

5.3.1 Tree K2, Bearing Fruitbodies of Lactarius pubescens

An 11-year-old birch tree (*B. pendula*, termed K2) in the experimental plot at the Bush Estate was selected, because in the autumn of 1981 the tree had been associated with fruitbodies of *L. pubescens*. The ground beneath the tree was mulched over winter to suppress the ground cover, as described earlier, and three areas around the tree were marked out on 13 June 1982 as shown in Figure 5.3. This was done by first marking six radii, separated by angles of 60°, from the tree. Points at 30 cm distance from the tree along each radius were joined by marked straight lines. This was repeated for points at 80 cm distance, such that six trapezium-shaped areas were marked around the tree. A narrow trench, 35 cm deep and 10 cm wide, was then dug around alternate areas in order to isolate the roots within those areas from any connection with the parent tree. Each trench was then lined with black polyethylene sheeting to act as a barrier to re-invasion of the trenched areas by roots or mycelial strands, and the centre part of the trench was refilled with soil. The experiment was designed such that three uniformly

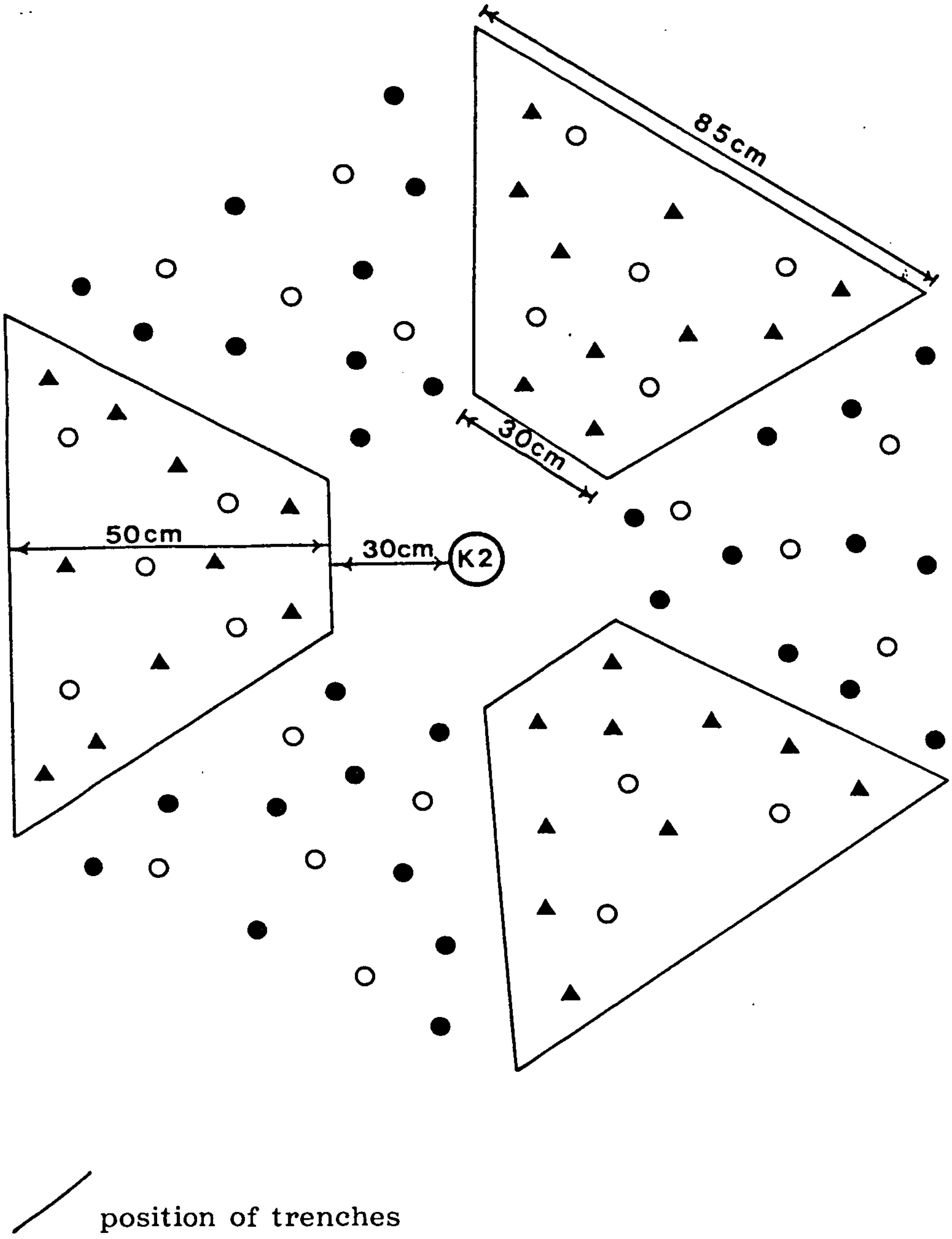


FIGURE 5.3: Distribution of seedlings planted in non-isolated (●), trenched (▲) and isolated (cored, O) positions around an 11-year old birch tree (K2) on the Bush Estate.

sized volumes of soil of approximately 0.1 m^3 and isolated from any contact with the parent tree alternated with similar areas containing attached tree roots.

Fifteen planting positions were selected in each of the six areas (Figure 5.3). It was intended to core five of the fifteen positions in each area and then immediately to replace the core as described in Section 5.2.1. Because of the presence of stones or large woody tree roots, however, a total of only twenty-six cores, evenly distributed between trenched and untrenched areas, could be cut. Both the trenching and the coring were done on 14 and 15 June 1982. A non-mycorrhizal birch seedling (Section 2.3.2) was planted into each position on 16 June. The seedlings were then 61 days old with a shoot height of 56 ± 3.9 (s.e.) mm and an oven-dry weight of 5.6 ± 1.16 mg, and they bore 45 ± 7.2 uninfected root tips. In all, thirty seedlings were grown in areas of attached tree roots (termed 'non-isolated'), thirty-one were grown within trenched areas (termed 'trenched') and twenty-six were grown in cores distributed between trenched and non-isolated areas (termed 'isolated') (Figure 5.3). The experimental procedure during the summer was the same as that described in the last section.

The seedlings were harvested on 26 October 1982, after 133 days' growth in the field; due to the unequal sample sizes (four seedlings had died in the non-isolated areas) comparisons between the treatments were based on the Student-Newman-Keuls test. There were no differences in shoot height between the treatments but the seedlings grown in the trenched and isolated positions were significantly heavier than in the non-isolated areas (Table 5.3.1). There was no significant difference in the mean total number of root tips between the treatments; there were, however, significantly more mycorrhizal tips on the non-isolated

TABLE 5.3.1: Mean shoot height and oven-dry weight of birch seedlings grown for 133 days in non-isolated, trenched and isolated (cored) positions around a mature birch tree (K2); data transformed to log(x+1) for analysis (in parentheses)*.

	Non-isolated (n=26)	Trenched (n=31)	Isolated (n=26)
Height (mm)	55 *(1.738)*	53 *(1.658)*	58 *(1.749)*
Dry weight (mg)	9.9^{a,b} *(1.009)*	14.8^{a} *(1.108)*	19.7^{b} *(1.144)*

*Figures followed by the same letter differ from one another at p=0.01.

TABLE 5.3.2: Mean number of root tips of different mycorrhizal types on birch seedlings grown in non-isolated, trenched and isolated (cored) positions around a mature birch tree (K2); data transformed to log(x+1) for analysis (in parentheses)*.

Mycorrhizal type	Non-isolated (n=26)	Trenched (n=31)	Isolated (n=26)
Lactarius	33^{b,c} *(1.167)*	3^{b} *(0.238)*	3^{c} *(0.227)*
Inocybe (I)	15 *(0.809)*	23 *(1.062)*	41 *(1.274)*
Laccaria (L)	2 *(0.062)*	7 *(0.251)*	4 *(0.136)*
Hebeloma (H)	3 *(0.288)*	1 *(0.089)*	5 *(0.282)*
Others (I,L,H)	20^{b,c} *(0.951)*	30^{b} *(1.358)*	50^{c} *(1.435)*
Uninfected root tips	7^{b,c} *(0.751)*	35^{a,b} *(1.425)*	29^{a,c} *(1.357)*
Total mycorrhizal tips	54^{a} *(1.632)*	34^{a} *(1.404)*	53 *(1.500)*
Total root tips	61 *(1.686)*	69 *(1.790)*	82 *(1.848)*

*Figures in each row followed by the same letter differ from one another at p=0.05 (a) or p=0.01 (b,c).

seedlings than on the trenched ones (Table 5.3.2). Uninfected root tips were present in significantly greater numbers on the 'trenched' than on the 'isolated' seedlings and in significantly greater numbers on the latter than on seedlings grown in the non-isolated positions (Table 5.3.2).

Three 'early-stage' mycorrhizal types (*sensu* Deacon *et al.*, 1983), namely Inocybe-type, Laccaria-type and Hebeloma-type, were seen on the seedlings. Individually, these types showed no significant difference between the treatments, but, grouped together, there were significantly more root tips of these types on the trenched and isolated seedlings than on the non-isolated ones. In contrast, there were significantly more mycorrhizas of *L. pubescens* on the non-isolated seedlings than on those in the other two categories (Table 5.3.2). In addition, the number of seedlings infected by *L. pubescens* differed between treatments: 20 of the 26 non-isolated seedlings were infected by *L. pubescens*, whereas only 7 of the 31 trenched seedlings and 6 of the 26 isolated seedlings were infected by this fungus. With respect to both the mean number of Lactarius-type mycorrhizas on the seedlings and the number of seedlings infected by *L. pubescens*, there was no difference between the trenched and isolated seedlings.

On 27 January 1983, nine soil cores (8 x 2 cm diameter) were collected, at random, within each of the six areas around the tree and examined for mycorrhizas. There was no difference between the trenched and non-isolated areas in the total number of mycorrhizal tips observed (Table 5.3.3); nevertheless, virtually all of the mycorrhizas in the trenched areas were moribund and were assumed to be dead (Section 2.5.2b). No live mycorrhizas of *L. pubescens* or *Hebeloma* spp. were observed in the trenched areas, whereas live mycorrhizal tips of all

TABLE 5.3.3: Mean numbers of mycorrhizal tips found in soil cores taken from trenched and non-isolated areas around a mature birch tree (K2) in January 1983; data transformed to log(x+1) for analysis (in parentheses).

Mycorrhizal type	Non-isolated (n=27)	Trenched (n=27)	LSD (p=0.05)
Lactarius	8	0	
	(0.632)	*(0.000)*	*(0.145)*
Inocybe	8	0	
	(0.752)	*(0.048)*	*(0.131)*
Laccaria	2	0	
	(0.170)	*(0.011)*	*(0.113)*
Hebeloma	1	0	
	(0.270)	*(0.000)*	*(0.260)*
Dead	7	21	
	(0.752)	*(1.251)*	*(0.119)*
Total	26	21	
	(1.325)	*(1.270)*	*(0.124)*

TABLE 5.3.4: Percentage of live mycorrhizal tips on birch seedlings and in soil cores* taken from around a mature birch tree (K2).

Mycorrhizal type	Seedlings			Soil cores*
	Non-isolated	Trenched	Isolated	Non-isolated
Lactarius	62.0	9.7	5.9	40.9
Hebeloma	6.5	1.6	8.5	7.1
Inocybe	28.5	67.9	77.1	43.1
Laccaria	2.9	20.8	8.4	8.9

* The cores were taken to sample the mycorrhizal types on the parent tree root system and were not planted with seedlings.

four types recorded on the seedlings were present in the non-isolated areas, and dead mycorrhizas formed only a small proportion of the total in these areas. Mycorrhizas of *L. pubescens* accounted for 40.9% of the live mycorrhizal tips of the parent tree roots in the non-isolated areas (Table 5.3.4); when this is compared with the percentage infection of the seedling root systems (Table 5.3.4), Lactarius-type mycorrhizas are seen to have been over-represented on the non-isolated seedlings (62.0%) and under-represented on the trenched (9.7%) and isolated (5.9%) seedlings. The other mycorrhizal types collectively showed the opposite pattern.

Seven fungal fruitbodies were recorded around the tree in 1982; all of these were of *L. pubescens* and all occurred in the non-isolated areas.

In summary, these results support those presented in Section 5.2.1, namely that *L. pubescens* readily colonises seedling roots provided that its inoculum is not severed from its connections with a mature tree; isolation of either large or small root volumes from the parent tree markedly reduces the degree of colonisation by *L. pubescens* of seedlings grown in such positions. The results are again consistent with the view that *L. pubescens* infected seedlings by mycelial strands or hyphae that needed to remain attached to a food base, a mature tree. The similarity in mycorrhizal colonisation between seedlings grown in trenched areas and soil cores, and the high proportion of dead roots in the trenched areas, indicates that trenching was probably successful in severing the majority of connections with the mature tree. Finally, adequate seedling growth seems to have occurred in the experiment, and seedlings grown in soil isolated from the mature tree were the heavier plants.

5.3.2 *Tree H5, Bearing Fruitbodies of Leccinum spp. and Lactarius glyciosmus*

The same experimental procedure as described in Section 5.3.1 but with some minor differences was followed in this experiment. Another tree (termed H5) in the experimental birch plot was selected, fruitbodies of *Leccinum scabrum*, *L. roseofracta* and *Lactarius glyciosmus* (Fr. ex Fr.) Fr. having been recorded around it in autumn 1981.

Trenches were dug around the tree as described earlier (Figure 5.3, Plate 5.1); however, sheets of corrugated 'Novolux' perspex were inserted into the trenches to act as a physical barrier to re-invasion of the trenched areas (Plate 5.2). The trenches were then refilled. The same number of planting positions as in the last experiment were chosen but it was again possible to core only twenty-six planting positions. Trenching and coring were done on 21 and 22 June 1982 and each position was planted with a non-mycorrhizal birch seedling on the following day, 23 June. The seedlings were taken from the propagators used in the last experiment but were now 67 days old.

The seedlings were harvested on 29 September, after 100 days' growth in the field. There was then no significant difference in the shoot height of the plants (Table 5.3.5), but the seedlings grown in the cores ('isolated') were significantly heavier than those grown in the trenched areas, which were, in turn, significantly heavier than those grown in the non-isolated areas (Table 5.3.5). There was no significant difference in either the total number of root tips or the total number of mycorrhizal root tips between the three treatments, although there were significantly more uninfected root tips on the isolated seedlings than on the trenched ones and significantly more on the latter than on the non-isolated ones (Table 5.3.6).

PLATE 5.1: A mature birch tree (H5) on the experimental birch plot on the Bush Estate showing the position of trenches dug around it.

PLATE 5.2: Distribution of seedlings and corrugated perspex barriers in trenches around a mature birch tree (H5).

TABLE 5.3.5: Mean shoot heights and oven-dry weights of birch seedlings grown for 100 days around a mature birch tree (H5) in non-isolated, trenched and isolated (cored) positions; data transformed to log(x+1) for analysis (in parentheses)*.

	Non-isolated (n=21)	Trenched (n=28)	Isolated (n=21)
Height (mm)	47 *(1.669)*	49 *(1.679)*	52 *(1.696)*
Dry weight (mg)	6.8^{ab} *(0.861)*	10.8^{ac} *(0.973)*	12.6^{bc} *(1.040)*

* Figures followed by the same letter differ significantly from one another at p=0.01.

TABLE 5.3.6: Mean numbers of root tips of different mycorrhizal types on seedlings grown around a mature birch tree (H5) in non-isolated, trenched and isolated (cored) positions; data transformed to log(x+1) for analysis (in parentheses)*.

Mycorrhizal type	Non-isolated (n=21)	Trenched (n=28)	Isolated (n=21)
Leccinum	36^{bc}	12^{bd}	3^{cd}
	(1.301)	*(0.640)*	*(0.086)*
Lactarius glyciosmus	11^{bc}	2^{b}	1^{c}
	(0.790)	*(0.182)*	*(0.100)*
Laccaria	1^{ab}	9^{ac}	23^{bc}
	(0.069)	*(0.437)*	*(0.693)*
Inocybe (I)	6	8	11
	(0.481)	*(0.294)*	*(0.377)*
Type 2 (BCT)	4	12	24
	(0.279)	*(0.548)*	*(0.779)*
Hebeloma (H)	1	3	2
	(0.150)	*(0.244)*	*(0.130)*
Cenococcum (C)	0	0	1
	(0.066)	*(0.055)*	*(0.141)*
Type 3 (3)	1	0	7
	(0.059)	*(0.000)*	*(0.258)*
Combined: I,BCT, H,C,3	13	24	45
	(0.866)	*(0.864)*	*(1.157)*
Uninfected	4^{bc}	13^{bd}	19^{cd}
	(0.579)	*(1.036)*	*(1.186)*
Total mycorrhizal root tips	61	48	72
	(1.654)	*(1.379)*	*(1.537)*
Total root tips	65	61	91
	(1.748)	*(1.672)*	*(1.795)*

* Figures in each row followed by the same letter differ from one another at p=0.05 (a) and p=0.01 (b,c,d).

A very high proportion of seedlings in the non-isolated areas were infected by Leccinum-type (19 out of 21) and Lactarius-type (15 out of 21) mycorrhizas. Many fewer 'trenched' (5 out of 28) and 'isolated' (3 out of 21) seedlings were colonised by Lactarius-type mycorrhizas. But whereas only one seedling grown in the cores had any Leccinum-type mycorrhizas, over half (16 out of 28) of the seedlings grown in the trenched areas were infected by this fungus. Nevertheless, there were significantly more mycorrhizal tips of *Leccinum* sp. on the non-isolated seedlings than on the trenched seedlings and significantly more on the latter than on the isolated seedlings (Table 5.3.6). In other words, the numbers of Leccinum-type mycorrhizas on the 'trenched' seedlings were intermediate between the numbers on the 'non-isolated' seedlings (on which Leccinum-type mycorrhizas were dominant) and on the 'isolated' seedlings (on which there was little infection by this fungus). There were also significantly more mycorrhizal tips of *Lactarius glyciosmus* on the non-isolated seedlings than on seedlings in the other treatments, but, unlike the results for Leccinum-type mycorrhizas, there was no significant difference in infection by *L. glyciosmus* between the trenched and isolated seedlings.

In contrast to the above results for presumed 'late-stage' fungi (*sensu* Deacon *et al.*, 1983), Laccaria-type mycorrhizas were present in significantly greater numbers on the isolated seedlings than on the trenched seedlings and in significantly greater numbers on the trenched seedlings than on the non-isolated ones. No other mycorrhizal type showed, individually, any significant difference between the treatments, nor did they do so if the remaining five types were combined (Table 5.3.6).

Nine soil cores (8 x 2 cm diameter) were collected at random in each area around the tree on 21 January 1983 and examined for mycorrhizas. Significantly more mycorrhizal tips were found in the non-isolated areas than in the trenched areas (Table 5.3.7) and, similarly, significantly more mycorrhizas of *L. glyciosmus*, *Leccinum* sp., *Inocybe* sp. and an unidentified type (type 3) were found in the non-isolated than trenched areas. The mycorrhizas of other types did not differ significantly in this respect (Table 5.3.7), perhaps because these mycorrhizal types were relatively scarce in both areas.

Several other features of interest in this experiment can be listed as follows:

1. Relatively low numbers of dead mycorrhizas were seen in the trenched areas, despite the marked reduction in total number of mycorrhizas in trenched compared with non-trenched areas. The simplest explanation of this is that many of the mycorrhizas that died must have degenerated to the extent that they were not recognisable.
2. The mycorrhizal type termed BCT (brown with cream tips, possibly formed by a member of the Ascomycotina) was present in surprisingly high numbers in soil cores even in the trenched areas.
3. No mycorrhizas of *Laccaria* sp. were seen in the soil cores, although this mycorrhizal type occurred on seedlings in all three treatments; likewise, no type 3 mycorrhizas were observed in the trenched areas although they occurred on seedlings in the trenched areas.
4. All three trenched areas were remarkably similar with regard to the occurrence of mycorrhizal types in the soil cores and on the seedlings grown in those areas.

TABLE 5.3.7: Mean numbers of mycorrhizal root tips on parent tree roots in cores taken from within trenched and non-isolated areas around a mature birch tree (H5) in January 1983; data transformed to log(x+1) for analysis (in parentheses).

Mycorrhizal type	Non-isolated (n=27)	Trenched (n=27)	LSD (p=0.05)
Leccinum	25 *(0.875)*	2 *(0.114)*	*(0.313)*
Lactarius	10 *(0.645)*	0 *(0.023)*	*(0.254)*
Inocybe (I)	11 *(0.831)*	2 *(0.172)*	*(0.260)*
Type 2 (BCT)	10 *(0.808)*	15 *(0.712)*	*(0.334)*
Hebeloma (H)	1 *(0.046)*	0 *(0.038)*	*(0.121)*
Cenococcum (C)	3 *(0.295)*	2 *(0.306)*	*(0.226)*
Type 3 (3)	6 *(0.373)*	0 *(0.000)*	*(0.214)*
[Combined: I,BCT H,C,3	30 *(1.342)*	19 *(0.933)*	*(0.253)*]
Dead	3 *(0.369)*	9 *(0.761)*	*(0.888)*
Total	68 *(1.659)*	30 *(1.253)*	*(0.258)*

TABLE 5.3.8: Mean percentage of mycorrhizas attributable to different fungi on seedlings grown in non-isolated, trenched and isolated (cored) positions around a mature birch tree (H5) and in soil in cores taken from non-isolated and trenched areas.

Mycorrhizal type	Seedlings Non-isolated	Seedlings Trenched	Seedlings Isolated	Cores Non-isolated	Cores Trenched
Leccinum	59.5	25.8	4.2	38.9	9.5
Lactarius	17.6	4.8	0.9	14.9	0.5
Laccaria	2.1	19.7	32.3	0.0	0.0
Inocybe	10.1	17.2	15.0	16.3	9.5
Type 2 (BCT)	6.9	25.0	32.9	15.5	69.5
Hebeloma	1.9	6.9	3.0	0.9	1.4
Cenococcum	0.5	0.5	1.2	4.2	9.5
Type 3	1.3	0.0	10.4	9.2	0.0

From Table 5.3.8 it is seen that Leccinum-type mycorrhizas were proportionally over-represented on the seedlings in non-isolated and trenched areas when compared with the proportion of Leccinum-type mycorrhizas found in soil cores from the respective areas; in contrast, Leccinum-type mycorrhizas were proportionally under-represented on seedlings grown in cored ('isolated') positions in the field. Lactarius-type mycorrhizas were present in similar proportions on the seedling and parent tree root systems in 'non-isolated' position but in the trenched areas this mycorrhizal type was proportionally over-represented on seedling roots. Most of the other mycorrhizal types were under-represented on the non-isolated seedlings and usually, but not always, over-represented on the trenched and cored seedlings (Table 5.3.8).

Two fruitbodies of *Leccinum scabrum*, four of *L. roseofracta* and nine of *Lactarius glyciosmus* were recorded around the tree in autumn 1982. One of the fruitbodies of *L. roseofracta* was recorded within a trenched area, 29 days after trenching (22 July); the other fruitbodies were all in non-isolated areas.

This experiment demonstrates that two other types of 'late-stage' fungus, *Leccinum* spp. and *L. glyciosmus*, colonised seedlings extensively when they were grown in the root region of a mature tree to which the inoculum of the fungus remained attached; but when the inoculum was isolated, in soil cores, then very little colonisation of seedlings occurred. As such, the results closely parallel those presented in Section 5.2.1 for *L. pubescens*, and they indicate again that mycelial strands or extramatrical mycelia~~l~~ may be important in initiating infection. However, the results for *Leccinum* spp. contrast with those for *Lactarius pubescens* with regard to colonisation of seedlings in trenched areas of soil. *Leccinum* spp. apparently were able to colonise seedling roots in trenched areas, though it is not clear if this was because a large isolated root volume provided a sufficient food base for infection or because the trenches did not completely isolate the soil within them from the mature tree.

5.4 Persistence of Mycorrhizas on Seedlings Transplanted into an Experimental Birch Stand

Some mycorrhizal fungi, when inoculated onto a seedling root system, are able to prevent or reduce subsequent colonisation of the roots by other mycorrhizal fungi (Section 4.2.1 and 4.3). The following experiment was done to see how a pre-established mycorrhizal fungus, *Thelephora terrestris*, would persist on roots of seedlings after these are transplanted into soil around a mature tree; additionally, a soil-coring treatment was used to isolate inocula of mycorrhizal fungi within the 'recipient' soil.

An area of approximately 1.5 m x 2.5 m within the experimental birch plot was selected, numerous fruitbodies of *Lactarius pubescens* having been observed in this area in autumn 1981. The ground was mulched with black polyethylene sheeting over the winter of 1981/82 to suppress the herbaceous ground cover. Meanwhile, two batches of birch seedlings were prepared: mycorrhizal with *T. terrestris* or non-mycorrhizal. The seedlings had been grown from aseptically germinated seeds sown in propagators on 16 April 1982 and they were 62 days old when transplanted into the field. The non-mycorrhizal seedlings were then both significantly taller and significantly heavier than their mycorrhizal counterparts, but there was no significant difference in the total number of root tips (Table 5.4.1).

TABLE 5.4.1: Mean shoot height and oven-dry weight and numbers of root tips on birch seedlings uninoculated or inoculated with *Thelephora terrestris* and incubated for 62 days; data transformed to log(x+1) for analysis (in parentheses).

Seedling inoculation	Height (mm)	Dry weight (mg)	No. mycorrhizal root tips	No. uninfected root tips	Total no. root tips
Thelephora	16 *(1.224)*	1.9 *(0.459)*	35	9 *(0.964)*	44 *(1.652)*
Controls	53 *(1.725)*	4.9 *(0.724)*	-	45 *(1.611)*	45 *(1.611)*
Significance of difference (p)	0.001	0.05	-	0.001	nil

Eighty planting positions were chosen, spaced 20 cm apart, in ten rows in a rectangular block (Figure 5.4). On 16 June, alternate planting positions were cored, as in previous experiments, and on 17 June 1982 the seedlings were planted into the final positions. Care was taken to ensure that each row of eight planting positions contained four mycorrhizal and four non-mycorrhizal seedlings, evenly distributed between cored and non-cored positions, but the locations of individual seedlings within the rows were random (Figure 5.4).

The seedlings were watered when necessary during the summer of 1982 and a peat mulch was applied, as before. The seedlings were harvested on 23 September after 106 days growth in the field, but many of them had not survived the summer and were discarded. The surviving seedlings were distributed between the treatments as follows: ten mycorrhizal (*T. terrestris*), cored; ten non-mycorrhizal, cored; thirteen mycorrhizal, non-cored; eighteen non-mycorrhizal, non-cored. To simplify comparisons only thirteen of the last category of seedlings were assessed.

As shown in Table 5.4.2, the originally non-mycorrhizal seedlings were significantly taller and heavier than those originally mycorrhizal with *T. terrestris*. Yet comparison of Tables 5.4.1 and 5.4.2 shows that the originally non-mycorrhizal seedlings made no height increment in the field, and similarly nor had the originally mycorrhizal seedlings. From the final dry weights (Table 5.4.2) it is seen that the originally mycorrhizal seedlings made little or no growth in the field, whereas the originally non-mycorrhizal seedlings had almost doubled their mean weight from the time of outplanting. The data for the mean total numbers of root tips at the end of the experiment (Table 5.4.3) show that the root systems of the originally non-mycorrhizal plants were similar to when they

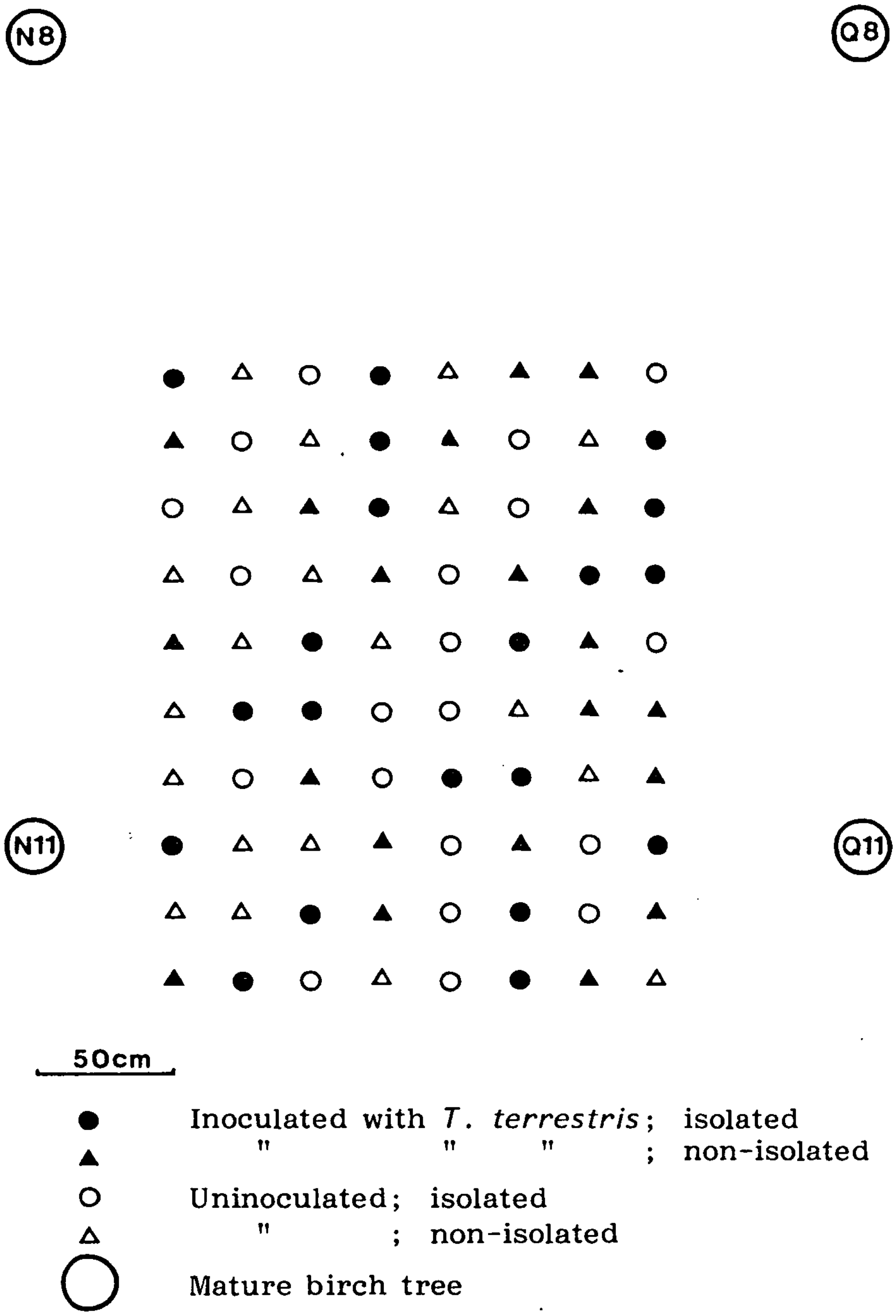


FIGURE 5.4: Positions, with respect to four trees, of seedlings inoculated with *Thelephora terrestris* or uninoculated and grown in isolated (cored) or non-isolated positions within an experimental birch stand.

TABLE 5.4.2: Mean shoot height and oven-dry weight of birch seedlings grown for 62 days in uninoculated vermiculite-peat or vermiculite-peat supplemented with *Thelephora terrestris* and then grown for 106 days in isolated (cored) or non-isolated positions within an experimental birch stand; data transformed to log(x+1), in parentheses, and analysed by 2-way analysis of variance.

	Seedling inoculation		
	Thelephora	None	Mean
Height (mm)			
Isolated	21 *(1.325)*	50 *(1.698)*	35^{b} *(1.512)*
Non-isolated	19 *(1.305)*	39 *(1.581)*	29^{b} *(1.443)*
Mean	20^{a}* *(1.314)*	44^{a} *(1.632)*	
Dry weight (mg)			
Isolated	1.9 *(0.461)*	9.2 *(0.939)*	5.6 *(0.700)*
Non-isolated	2.1 *(0.476)*	8.1 *(0.867)*	5.1 *(0.672)*
Mean	2.0^{c} *(0.470)*	8.6^{c} *(0.898)*	

* Figures followed by the same letter differ from one another at p=0.05 (b) or p=0.001 (a,c); the analysis of variance showed no significant interaction between inoculation and coring treatments.

were transplanted into the field, whereas the originally mycorrhizal plants showed a substantial reduction in the numbers of root tips. From all these results and from the death of some seedlings it must be concluded that conditions in the field were unsatisfactory for seedling growth, and caution is therefore needed in interpreting the mycorrhizal distributions outlined below.

Isolation of the seedlings in cores had no effect on the occurrence of any mycorrhizal type although there were significantly more uninfected root tips in isolated than in non-isolated planting positions (Table 5.4.3). *T. terrestris* was evidently able to persist on the root systems on which it was initially present (Table 5.4.3), and in its absence (i.e. on the control seedlings) the root systems became colonised by other mycorrhizal types, including Laccaria-type (Table 5.4.3) and the mycorrhizas of *Hebeloma* spp., *Inocybe* spp., *Lactarius pubescens* and *Cenococcum geophilum*, which are grouped together as 'others' (Table 5.4.3). In fact, 85.3% of the total number of root tips on the control seedlings were infected by mycorrhizal types other than Thelephora-type, whereas the corresponding figure for the seedlings initially inoculated with *T. terrestris* was only 42.5%. Again, however, it must be stressed that the experiment did not provide a critical test of the ability of *T. terrestris* to persist on and colonise seedling roots in the field, because there was no net root production during the period of the experiment.

Many fruitbodies were seen within the experimental area while the seedlings were growing; they were assigned to the species *Laccaria tortilis* (34 fruitbodies), *Lactarius pubescens* (5 fruitbodies) and *Russula grisea* (2 fruitbodies).

TABLE 5.4.3: Mean numbers of root tips of different mycorrhizal types on birch seedlings, non-mycorrhizal or mycorrhizal with *Thelephora terrestris* and grown for 106 days in isolated (cored) or non-isolated positions within an experimental birch stand; data transformed to log(x+1), in parentheses, and analysed by 2-way analysis of variance.

Mycorrhizal type	Soil treatment	Seedling inoculation		
		Thelephora	None	Mean
Thelephora	Isolated	12 *(0.986)*	0 *(0.000)*	6 *(0.493)*
	Non-isolated	8 *(0.716)*	1 *(0.185)*	5 *(0.450)*
	Mean	10^{a*} *(0.833)*	1^{a} *(0.104)*	
Laccaria	Isolated	2 *(0.207)*	12 *(0.776)*	7 *(0.492)*
	Non-isolated	1 *(0.155)*	12 *(0.800)*	7 *(0.478)*
	Mean	2^{b} *(0.178)*	12^{b} *(0.789)*	
'Others'	Isolated	6 *(0.918)*	24 *(1.329)*	15 *(0.873)*
	Non-isolated	9 *(0.980)*	17 *(1.125)*	13 *(0.891)*
	Mean	7^{c} *(0.708)*	20^{c} *(1.058)*	
Uninfected	Isolated	3 *(0.436)*	7 *(0.876)*	5^{f} *(0.656)*
	Non-isolated	3 *(0.369)*	3 *(0.495)*	3^{f} *(0.432)*
	Mean	3^{d} *(0.399)*	5^{d} *(0.661)*	
Total root tips	Isolated	23 *(1.296)*	44 *(1.594)*	33 *(1.445)*
	Non-isolated	21 *(1.276)*	37 *(1.463)*	30 *(1.369)*
	Mean	22^{e} *(1.285)*	40^{e} *(1.520)*	

*Figures followed by same letter differ from one another at p=0.05 (c,d,e,f) or p=0.001 (a,b); the analysis of variance showed no significant interaction between inoculation and coring treatments.

5.5 Mycorrhizal Development on Seedlings Grown in Woodland Soils

The work described so far in this chapter has involved investigations into the mycorrhizal status of seedlings transplanted into an experimental stand of birches established relatively recently on former agricultural soil. It was considered of interest to see whether the results from those experiments were supported by evidence from natural or man-managed woodlands, especially on sites that may have had a long history of continuous tree cover. Two contrasting woodland sites were chosen for investigation, the two main objectives being as follows.

1. To establish whether the broad distinction between 'early-stage' and 'late-stage' mycorrhizal types (Deacon *et al.*, 1983) is applicable to such 'natural' woodland habitats.
2. If so, then to assess the occurrence of 'late-stage' fungi on naturally regenerating birch in woodland environments.

5.5.1 Bridge Wood, Rochester, Kent

This woodland (OS reference TQ 739637) is a managed sweet chestnut (*Castanea sativa* Mill.) coppice with oak standards on a 'clay with flints' soil overlying chalk. The site was first visited in 1981 when an area of coppice had recently been cut. There were numerous naturally regenerating birch seedlings growing on and around the chestnut stools, consistent with the report by Ford and Newbould (1977) that seedlings of *B. pubescens* form a significant part of the ground flora of chestnut coppice woodlands in the first few years after coppicing. Investigations into the site in 1981 and 1982 are now described.

(a) Investigations in 1981

Naturally regenerating birch seedlings and the soil in which they were growing were sampled from six locations within the coppice on 27 July 1981. The samples were collected from the following locations.

1. Soil and leaf litter in the rotten centre of a coppice stool (stump 1); pH 4.2.
2. Soil within a 50 cm radius of stump 1 (excluding site 3); pH 3.9.
3. Soil from a steep sandy bank adjacent to stump 1; pH 4.3.
4. Soil and leaf litter within the rotten centre of a second chestnut stool (stump 2); pH 3.9.
5. Soil within a 50 cm radius of stump 2; pH 4.6.
6. Soil within 1 m distance of an oak standard; pH 4.6.

The seedlings were preserved in 2% glutaraldehyde solution before being taken to Edinburgh for examination. Soil from each location was used to fill eight 5.0 cm square pots, each of which was then planted with an aseptically germinated birch seedling on 11 August 1981. The pots were randomised in two propagator trays and placed in a growth cabinet (Section 2.2.3) until the plants were sufficiently well established to be transferred to a heated glasshouse, 38 days later (18 September). The seedlings were harvested on 18 December after 139 days' growth and assessed for mycorrhizal development.

There was no significant difference in the heights and leaf areas of the seedlings collected in the field; overall they were 21 ± 1.1 mm tall and had a leaf area of 261 ± 33.4 mm^2. All the seedlings were assumed to be less than one-year-old, as was later confirmed by sectioning. Forty-one of the total sixty-nine seedlings (59.4%) collected at the site were

TABLE 5.5.1: Mean numbers (± s.e.) of root tips of different mycorrhizal types on seedlings collected from six locations within a chestnut coppice in July 1981.

Mycorrhizal types on seedling roots	Location (n = no. of seedlings examined)*						Overall mean
	Coppice stool 1			Coppice stool 2		Oak standard	
	Soil within stump (n=7)	Soil within 50 cm radius (n=6)	Soil from steep bank (n=16)	Soil within stump (n=7)	Soil within 50 cm radius (n=12)	Soil within 1 m radius (n=21)	(n=69)
Type 1	5 ± 4.5	13 ± 7.1	10 ± 3.1	19 ± 4.7	11 ± 1.9	2 ± 0.9	8 ± 1.3
Cenococcum	2 ± 1.1	0	5 ± 1.5	5 ± 1.7	4 ± 1.9	0	3 ± 0.6
Type 12	5 ± 3.1	0	4 ± 3.0	4 ± 3.6	5 ± 1.4	5 ± 1.4	4 ± 1.0
Type 13	3 ± 1.9	0	6 ± 2.3	0	0	6 ± 1.5	4 ± 0.8
Type 14	0	4 ± 1.7	6 ± 2.1	0	0	2 ± 1.7	2 ± 0.8
Uninfected	43 ± 7.3	13 ± 3.4	15 ± 2.0	17 ± 2.6	10 ± 1.5	8 ± 0.6	15 ± 1.5

* See text for details.

TABLE 5.5.2: Mean shoot heights and leaf areas of birch seedlings grown for 139 days in pots of soil collected from six locations in a chestnut coppice; data transformed to log(x+1) for analysis (in parentheses).

	Origin of soil (see text)						
	Coppice stool 1			Coppice stool 2		Oak standard	LSD
	Within stump	50 cm radius	Steep bank	Within stump	50 cm radius	1 m radius	(p=0.05)
Height (mm)	87	38	19	83	66	73	
	(1.940)	*(1.585)*	*(1.272)*	*(1.920)*	*(1.825)*	*(1.869)*	*(0.087)*
Leaf area (mm^2)	2139	747	74	1444	1885	1663	
	(3.276)	*(2.848)*	*(1.549)*	*(3.127)*	*(3.259)*	*(3.216)*	*(0.228)*

TABLE 5.5.3: Mean numbers (± s.e.) of root tips of different mycorrhizal types on seedlings grown for 139 days in pots of soil from a chestnut coppice.

Mycorrhizal types on seedling roots	Origin of soil (see text)						Overall mean
	Coppice stool 1			Coppice stool 2		Oak standard	
	Soil within stump	Soil within 50 cm radius	Soil from steep bank	Soil within stump	Soil within 50 cm radius	Soil within 1 m radius	
Type 1	0	0	0	0	0	0	0
Cenococcum	127 ± 19.0	19 ± 5.8	2 ± 1.6	68 ± 13.7	22 ± 6.9	47 ± 9.1	48 ± 7.4
Type 12	0	0	0	0	0	0	0
Type 13	0	0	0	12 ± 7.9	11 ± 8.2	18 ± 8.5	7 ± 2.5
Type 14	24 ± 15.1	28 ± 12.7	0	13 ± 5.7	45 ± 6.5	40 ± 7.5	25 ± 4.3
Type 15	0	3 ± 2.5	9 ± 4.3	1 ± 0.9	0	8 ± 2.1	3 ± 1.5
Paxillus	20 ± 10.5	13 ± 9.5	0	16 ± 9.2	3 ± 6.4	0	9 ± 2.9
Uninfected	49 ± 6.6	21 ± 3.3	40 ± 3.1	48 ± 6.5	37 ± 4.5	34 ± 12.2	38 ± 2.3

infected by a distinct mycorrhizal type (type 1) that formed thick yellow mycelial strands. However, only six of the twenty-one seedlings collected from around the oak standard (location 6 above) were infected by this fungus, whereas 73% of the other seedlings (locations 1, 2, 3, 4 and 5) bore it. The dominance or co-dominance of this mycorrhizal type on seedlings in or around the chestnut stumps is shown in Table 5.5.1. Four other mycorrhizal types were seen on the seedlings collected from the site, as also shown in Table 5.5.1.

When incubated in the glasshouse, the artificially established seedlings grew to different degrees in the soils from different locations in the coppice (Table 5.5.2), the largest seedlings being those grown in soil from the rotten centres of the two stumps and in the soil from around the oak standard. Five mycorrhizal types occurred on the seedlings in the glasshouse, but only three of these types had been observed on seedlings collected from the field and, of most interest, type 1, which was most common in the field, was absent from the glasshouse-grown seedlings (Table 5.5.3).

Although these results must be treated with caution, they nevertheless suggest that mycorrhizal fungi that are able to colonise naturally regenerating seedlings in field sites do not colonise seedling roots (in a glasshouse) when the soil is removed from a field site in which the soil-borne inoculum may be attached to a mature tree.

(b) Investigations in 1982

The woodland was visited again in the following year, but unfortunately the stony nature of the site and its distance from Edinburgh precluded experiments such as those described in Section 5.2. The main aims of the work in 1982 were therefore as follows.

1. To repeat the experiment of the previous year, namely to compare the mycorrhizas found on naturally occurring seedlings in the woodland with those that develop on seedlings grown in the woodland soil in a glasshouse.
2. To collect seedlings from the field and to investigate the spread of the naturally established mycorrhizal fungi when the seedlings were transplanted into pots of soil in a glasshouse; in particular, it was hoped to see whether the existing mycorrhizal fungi on seedlings differed in their ability to colonise newly emerging roots.
3. To introduce laboratory-grown seedlings with established 'early-stage' mycorrhizal types into the coppice, and concurrently into pots of coppice soil in a glasshouse, in order to assess the abilities of such 'early-stage' mycorrhizal fungi to restrict natural colonisation of seedling roots by indigenous and possibly 'late-stage' mycorrhizal fungi.

Forty birch seedlings were collected from two sites within the coppice on 5 July 1982, the sampling locations being in the same area as was studied in 1981. Unfortunately, half the seedlings died in transit, all of these having come from one of the sites. It was therefore necessary to use the surviving seedlings for transplanting into soil as well as to provide information on the mycorrhizal status of seedlings in the woodland. They were thus assessed non-destructively for mycorrhizal infection after careful washing of their roots and with as little damage to the root systems as possible. The seedlings were all in their second growing season and were correspondingly taller (7.2 ± 0.46 cm) than the seedlings collected in 1981 (2.1 ± 0.11 cm). Type 1 mycorrhizas were present on all of the seedlings examined and, as in the 1981 sampling, were the dominant mycorrhizal type (Table 5.5.4); with the exception of mycorrhizal type 7 and the mycorrhizas of *Thelephora terrestris*, the seedlings were infected by a similar range of mycorrhizal types as in the previous year (c.f. Tables 5.5.1 and 5.5.4).

The seedlings were then transplanted on 8 July 1982 into 10 cm diameter round pots containing either 'non-mycorrhizal' soil collected from the Bush Estate (Section 2.4.2) or soil from the coppiced area. Ten replicate seedlings, randomly selected, were planted in each soil. The pots were then randomised and incubated in a heated glasshouse for 130 days, before sampling on 15 November 1982. As shown in Table 5.5.5 the mean height increment of the seedlings grown in coppice soil was over twice that of the seedlings grown in Bush soil, even though the seedlings randomly selected for transplanting into coppice soil were initially slightly the smaller.

As shown in Table 5.5.4, after 130 days growth in a glasshouse there was a marked decrease in the mean numbers of mycorrhizal types 1, 13 and 15 on the seedlings, compared with the numbers of these mycorrhizal types present initially, and this occurred irrespective of the soil in which the seedlings were grown. In fact, only 3 of 10 seedlings in each soil bore type 1 mycorrhizas at the final sampling, although this mycorrhizal type was originally present on all of the seedlings; there was an increase in numbers of this type on only one seedling out of the twenty examined. Type 15 mycorrhizas, originally present in relatively high numbers, were absent from all seedlings after 130 days, as were types 12 and 13 in the non-mycorrhizal Bush soil (Table 5.5.4). Of the mycorrhizal types originally present on the seedlings, only Cenococcum-type and Thelephora-type increased in numbers in both soils; type 12 increased in numbers in the coppice soil and type 7 increased in numbers

TABLE 5.5.4: Mean numbers (± s.e.) of root tips of different mycorrhizal types on seedlings collected from a chestnut coppice on 5 July 1982, and on the same seedlings after 130 days' growth in pots of 'Bush soil' or 'Coppice soil' in a glasshouse.

Mycorrhizal type	Sampling time (days) 0 (freshly collected seedlings)	130 Bush soil	 Coppice soil
Type 1	59 ± 14.3	29 ± 20.1	10 ± 9.3
Cenococcum	21 ± 14.9	164 ± 71.4	181 ± 73.3
Type 12	34 ± 17.5	0	60 ± 59.8
Type 13	37 ± 16.9	0	1 ± 1.0
Type 15	19 ± 6.2	0	0
Thelephora	21 ± 10.5	464 ± 227.5	425 ± 187.6
Type 7	5 ± 5.0	56 ± 47.9	7 ± 6.6
Type 11	0	36 ± 23.9	98 ± 93.7
Type 16	0	296 ± 161.4	236 ± 114.3
Paxillus	0	25 ± 10.9	222 ± 78.2
Laccaria	0	403 ± 209.2	51 ± 47.7
Hebeloma	0	312 ± 142.1	0
Others	0	9 ± 9.1	204 ± 140.7
Uninfected	0	128 ± 33.9	85 ± 12.2
Total	197 ± 35.8	1922 ± 127.2	1580 ± 138.9

TABLE 5.5.5: Mean shoot heights (cm, ± s.e.) of naturally regenerated birch seedlings at the time of transplanting into two soils, and their mean height increments and final heights after 130 days' growth in the soils in a glasshouse.

Soil	Transplanting	After 130 days	Mean increment
Bush	8.0 ± 0.63	18.0 ± 1.20	10.0 ± 0.90
Coppice	6.5 ± 0.61	27.5 ± 1.67	21.0 ± 1.42

in the non-mycorrhizal soil (Table 5.5.4). Five other mycorrhizal types that were not originally present on the seedlings were observed after 130 days' growth; they accounted for 56.2% of the total number of root tips in the non-mycorrhizal soil and 51.4% of the root tips in the coppice soil. A fruitbody of *Peziza badia* appeared in one of the pots of Bush soil on 8 November, but only two mycorrhizal types occurred on the seedlings in this pot and they were both attributable to the Basidiomycotina as evidenced by the presence of clamp connections.

All the named mycorrhizal types observed in this experiment are known to be 'early-stage' *sensu* Deacon *et al.* (1983); to this category must be added types 11 and 16, in that they readily colonised the new actively growing seedling roots. It is possible that the mycelial strand-forming type 1 mycorrhizas represent an unidentified 'late-stage' fungus but the status of the other types is unknown and will be discussed later.

The soil collected in Kent was used also for a seedling bioassay. Ten 10 cm round pots were filled with the coppice soil and five with 'non-mycorrhizal' Bush soil. Into each pot was planted a non-mycorrhizal birch seedling on 9 July 1982, the seedlings having been grown in propagators (Section 2.3.2) and being 41 ± 3.8 mm tall and of 2.6 ± 0.27 mg oven-dry weight. The pots were then incubated in a heated glasshouse (max. 28°C, min. 14°C) and sampled 116 days later. At sampling, the shoots of seedlings in coppice soil were 7.6 ± 0.49 cm tall and weighed 206 ± 26.5 mg, whereas those in Bush soil were 6.7 ± 0.74 cm tall and were of 159 ± 24.4 mg dry weight. Thelephora-type mycorrhizas were the dominant type on seedlings grown in Bush soil, the only other type present being attributable to *Paxillus involutus* (Table 5.5.6). On the seedlings grown in coppice soil the mycorrhizas of *Thelephora terrestris* and *P. involutus* were co-dominant, but Cenococcum-

TABLE 5.5.6: Mean numbers (± s.e.) of root tips of different mycorrhizal types on birch seedlings grown for 116 days in 'non-mycorrhizal' Bush soil or in coppice soil.

Soil	Paxillus	Cenococcum	Thelephora	Others	Uninfected	Total
Bush	69 ± 44.3	0	555 ±105.9	0	31 ± 8.4	653 ± 115.3
Coppice	188 ± 56.3	92 ±29.9	173 ± 45.6	2 ± 2.5	69 ± 17.4	525 ± 59.3

type mycorrhizas were also present in relatively large numbers. Interestingly, the seedling roots were not infected by any other mycorrhizal fungus that commonly occurred in the woodland site (c.f. Tables 5.5.4 and 5.5.6).

Before the coppice site was visited in July 1982, birch seedlings had been grown for 70 days in a growth room in cores of soil (8 x 2 cm diameter collected from in or around the experimental birch plot at Farfield (Section 3.2). The cores were of two types: (1) from areas where the fruitbodies of *Laccaria tortilis* had been observed to be abundant and (2) from nearby areas at least 10 m away from any trees. Twenty-one seedlings in each type of core - 'mycorrhizal' (with *Laccaria*) and 'controls' - were taken to the coppice site for transplantation. Two small clearings of about 5 m diameter in the coppice were selected, these being surrounded by at least six chestnut stools and an oak standard. All naturally regenerating birch seedlings were removed from the areas and seven replicate seedlings in each type of core were planted into each site on 5 July. The seedlings were planted in a block with 30 cm spacing between them and the planting positions of the two treatments were alternated. Planting was done by making a small hole in the ground, just sufficient to accommodate the soil core and with minimal disturbance to the surrounding soil. Seven replicates of each batch of seedlings were not transplanted; three of them were sampled immediately and assessed for mycorrhizas, and the remaining four were planted on 8 July into soil from the coppice site and were grown in a heated glasshouse for 116 days before being harvested. The seedlings planted into the coppice site were sampled on 17 September 1982, but only eight plants had survived the summer; these were all from one of the planting sites.

The seedlings grown in pots in the glasshouse were considerably larger than the seedlings grown in the coppice (Table 5.5.7); in fact there was no significant increase, from the time of transplanting, in the height of the surviving seedlings in the coppice. At the time of transplanting Laccaria-type mycorrhizas were present on seedlings grown in both the 'mycorrhizal' (*Laccaria*) cores and the control cores, but in significantly greater numbers on the 'mycorrhizal' seedlings (Table 5.5.7). After 116 days' growth in coppice soil in a glasshouse the seedlings bore a further five mycorrhizal types: Thelephora-type, Cenococcum-type, Paxillus-type, Hebeloma-type and Inocybe-type. These types represented the majority of mycorrhizal tips formed on the seedlings in the glasshouse (Table 5.5.7); indeed, only one seedling in each type of core had any Laccaria-type mycorrhizas, and there was no difference in infection by Laccaria-type mycorrhizas between the treatments. In contrast, mycorrhizas similar to those seen on naturally regenerating seedlings in the woodland were present, and dominant, on the seedlings transplanted into the field site; the mycorrhizal types did not, however, include types 1 and 12. There were very few Laccaria-type mycorrhizas on seedlings retrieved from the field and their numbers did not differ between the treatments (Table 5.5.7). Interpretation of these results is made difficult because of the poor survival of seedlings in the field and the differences in growth and mycorrhizal colonisation between glasshouse and field-grown seedlings. From the limited evidence, however, it seems that Laccaria-type mycorrhizas do not readily persist on seedling root systems after transplanting, and the new roots consequently are infected by naturally occurring mycorrhizal fungi.

The results from this investigation overall show again that some mycorrhizal fungi that are present on naturally regenerating birch growing

TABLE 5.5.7: Mean shoot height and dry weight and numbers of root tips on seedlings grown initially in soil cores from under fruitbodies of *Laccaria* sp. or from tree-less areas (controls) and transplanted into coppice soil in a glasshouse for 116 days or into a coppice woodland site for 72 days.

	Seedling treatment	Height (mm)	Dry weight (mg)	Mycorrhizal types		Total root tips
				Laccaria	Others	
At trans-planting	*Laccaria* (n=3)	45 ± 7.2	14.1 ± 3.41	71 ± 13.2	0	22 ± 12.2
	Control (n=3)	36 ± 3.2	17.6 ± 4.96	12 ± 12.3	0	47 ± 23.0
Glasshouse-grown plants	*Laccaria* (n=4)	117 ± 3.2	465.6 ± 48.70	230 ± 230.0	727 ± 364.1	1097 ± 113.2
	Control (n=3)	109 ± 9.8	442.1 ± 52.50	255 ± 255.0	815 ± 211.9	1197 ± 21.3
Coppice-grown plants	*Laccaria* (n=4)	55 ± 5.1	-	13 ± 8.1	78 ± 36.4	121 ± 31.9
	Control (n=4)	44 ± 4.5	-	12 ± 6.9	44 ± 4.0	65 ± 15.5

around coppice stools do not colonise seedlings that are grown in the same soil in a glasshouse. The fungi include the mycorrhizal type (type 1) that was dominant on seedlings collected in the field. However, the results must be treated with caution because some types, namely types 12, 13 and 15, that colonised glasshouse-grown seedlings in an experiment a year earlier did not do so in the subsequent experiment. Likewise, many mycorrhizal types present on seedlings in the field did not spread with the growing root system after the seedlings were transplanted to pots of soil in a glasshouse, but other types, not seen on seedlings in the woodland, became the predominant types on the root systems in glasshouse conditions. It is possible that the different environmental conditions in a glasshouse compared with in the field resulted in establishment of different mycorrhizal types in these environments; equally likely is the possibility that the inoculum sources were different in the two environments, especially as the glasshouse studies necessarily involved severence of inoculum in the soil from attachment to a parent tree.

5.5.2 *Struan Wood, Calvine, Perthshire*

This relict, ancient birch woodland (OS reference NN 7966) has been designated as a Site of Special Scientific Interest by the Nature Conservancy, as the site is believed to have had continuous woodland cover since the last Ice Age. The woodland now consists almost exclusively of old scattered birch trees; although there are numerous birch seedlings and dwarf older plants in the ground vegetation, the woodland is very heavily grazed by sheep with the result that these regenerated plants are rarely more than a few centimetres tall. There is thus a marked imbalance in the size class structure of the woodland. The soil was not analysed in detail but seemed to be intermediate between an acid brown earth and a podzol, and it had a deep (10 cm) surface accumulation of mor humus of pH 4.2 - 4.4. Making use of this site it was decided to repeat, and extend, the work of Deacon *et al.* (1983) by comparing the mycorrhizas found in the soil beneath the fruitbodies of mycorrhizal fungi with the mycorrhizas that developed on seedlings grown in the same soils. It was also intended to compare the mycorrhizas formed on naturally regenerating birch seedlings in the root zone of mature trees with the mycorrhizas formed on seedlings grown in the same soils in a glasshouse.

Composite samples of soil, humus and plant litter were collected on 1 October 1982 from beneath the fruitbodies of *Laccaria laccata*, *Lactarius tabidus* Fr., *Leccinum roseofracta*, *Amanita muscaria* and a *Cortinarius* sp. Only one group of naturally regenerating birch that appeared to be less than one-year-old could be found - in the litter layer on a hummock around a mature tree. These seedlings and the soil in which they were growing were also sampled, and the seedlings were examined for mycorrhizas. The soil was taken with a trowel from beneath each type of fruitbody, care being taken to keep the soil ball intact and it was then divided into two equal portions. One portion was washed over a sieve and the residue was assessed for mycorrhizas (Section 2.5.2). The remaining portions of soil, and the soil from the seedling regeneration site, were used to fill 5.0 cm square pots, each of which was planted with an aseptically germinated birch seedling on 17 October 1982. The numbers of replicate pots varied with the volume of soil available. The pots were randomised and incubated in a heated glasshouse for 141 days, being harvested on 7 March 1983.

There was then no significant difference in the shoot heights of the seedlings in the different soil samples but there were significant

TABLE 5.5.8: Mean shoot heights and dry weights of seedlings grown in a glasshouse for 141 days in soil collected from beneath fruitbodies of five different fungi and from one site of natural regeneration in Struan Wood; data transformed to log(x+1) for analysis (in parentheses).

	Origin of soil						
	Amanita muscaria (n=11)	*Leccinum roseofracta* (n=5)	*Cortinarius* sp. (n=17)	*Lactarius tabidus* (n=20)	*Laccaria laccata* (n=13)	Regeneration site (n=16)	LSD (p=0.05)
Height (mm)	21 *(1.334)*	23 *(1.374)*	18 *(1.269)*	19 *(1.287)*	22 *(1.346)*	21 *(1.315)*	*(0.202)*
Dry weight (mg)	18.0 *(1.161)*	25.4 *(1.359)*	14.9 *(1.095)*	13.4 *(1.081)*	22.2 *(1.268)*	19.1 *(1.245)*	*(0.139)*

TABLE 5.5.9: Mean numbers (± s.e.) of mycorrhizal tips on naturally regenerating birch seedlings from a site in Struan Wood and on seedlings grown in a glasshouse in soil from around the regenerating seedlings.

	Mycorrhizal type			
Seedling type	*Elaphomyces*	*Cenococcum*	Type 10	Uninfected
Naturally regenerating (n=20)	20 ± 2.5	1 ± 0.5	4 ± 1.1	0
Glasshouse-grown (n=16)	46 ± 3.7	1 ± 0.6	1 ± 0.5	29 ± 1.9

differences in shoot dry weight for reasons that were not investigated (Table 5.5.8). All twenty of the seedlings collected from the woodland were infected by a dominant mycorrhizal type (Table 5.5.9); this was identified as the mycorrhiza of *Elaphomyces granulatus* Fr., as several fruitbodies of this fungus were present and they were completely encrusted by this mycorrhizal type. All sixteen of the seedlings grown in the same soil in the glasshouse were also heavily colonised by this mycorrhizal type; in fact, there was little difference in either the range or proportions of mycorrhizal types on the root systems of glasshouse-grown seedlings compared with on the naturally regenerated seedlings from this localised site (Table 5.5.9).

The mycorrhizas of *L. roseofracta* and *Amanita muscaria* were always found in the soil beneath their fruitbodies (Table 5.5.10) and were always the dominant types; indeed, these mycorrhizal types were present to the almost complete exclusion of others. Lactarius-type mycorrhizas, similarly, were always present beneath the fruitbodies of *L. tabidus* but they were not always dominant and four other mycorrhizal types, including Leccinum-type, were also recorded. Type 3 mycorrhizas were found only beneath the fruitbodies of the *Cortinarius* sp.; these mycorrhizas are similar to some reported descriptions of Cortinarius-type mycorrhizas (Zak, 1969b) and in this investigation type 3 mycorrhizas are assumed to represent Cortinarius-type. This type was not present beneath all of the fruitbodies of *Cortinarius* sp. and was not always dominant even when it occurred. Similarly, Laccaria-type mycorrhizas were not always present beneath the fruitbodies of *L. laccata* and were never dominant, a wide range of other mycorrhizal types being found beneath the fruitbodies of *Cortinarius* sp. and *L. laccata*.

TABLE 5.5.10: Summary of the occurrence of mycorrhizal types in soil collected from beneath the fruitbodies of five mycorrhizal fungi in Struan Wood and the mycorrhizas found on seedlings grown in the same soils in a glasshouse.

Fruitbody beneath which soil was collected	No. soil samples	No. with mycorrhizas of fruitbody type	No. with predominance of mycorrhizas of fruitbody type	Other mycorrhizal types present (no. of soil samples with each type in parentheses)
Amanita muscaria	4	4	4	*Elaphomyces* (1); *Cenococcum* (1).
Leccinum roseofracta	3	3	3	None
Lactarius tabidus	8	8	4	*Leccinum* (4); *Cenococcum* (4); type 10 (3); *Hebeloma* (1).
Cortinarius sp.	11	7	5	*Cenococcum* (10); *Lactarius* (7); type 10 (4); type 17 (2); *Paxillus* (2); *Amanita* (2); *Leccinum* (1); *Laccaria* (1).
Laccaria laccata	6	4	0	*Leccinum* (4); *Cenococcum* (4); *Lactarius* (3); *Amanita* (1); *Paxillus* (1); type 10 (1).

	No. seedlings examined	No. seedlings with mycorrhizas of fruitbody type	No. seedlings with predominance of mycorrhizas of fruitbody type	Other mycorrhizal types present (no. seedlings infected by each type in parentheses)
Amanita muscaria	11	0	0	*Cenococcum* (8); *Laccaria* (7); *Leccinum* (3); Inocybe (3); *Lactarius* (3); type 17 (2).
Leccinum roseofracta	5	0	0	Type 10 (5); *Lactarius* (3); type 17 (3); *Cenococcum* (2); *Cortinarius* (1); *Elaphomyces* (1).
Lactarius tabidus	20	18	12	Type 17 (8); *Cenococcum* (7); *Paxillus* (4); *Leccinum* (3); type 8 (2); type 9 (2); *Hebeloma* (2); *Thelephora* (1); *Amanita* (1); type 18 (1).
Cortinarius sp.	17	7	4	*Cenococcum* (14); type 17 (9); *Inocybe* (7); *Leccinum* (3); *Paxillus* (3); *Lactarius* (2); *Laccaria* (2); *Elaphomyces* (2); *Amanita* (1); type 8 (1); type 9 (1); type 18 (1).
Laccaria laccata	13	5	4	*Cenococcum* (9); type 8 (7); *Inocybe* (6); *Lactarius* (4); *Paxillus* (2); type 3 (2); *Leccinum* (1); *Elaphomyces* (1); type 10 (1).

Seedlings grown in soil taken from beneath the fruitbodies of *A. muscaria* did not develop mycorrhizas typical of this fungus (Table 5.5.10). Likewise, no Leccinum-type mycorrhizas were formed on seedlings grown in soil taken from beneath the fruitbodies of *L. roseofracta*. Overall, however, 12 out of 66 seedlings (excluding those from the natural regeneration site) were colonised by these two mycorrhizal types, and on 7 glasshouse-grown seedlings these types were dominant. Nearly all (18 out of 20) of the seedlings grown in soil from beneath the fruitbodies of *L. tabidus* were colonised by this fungus and it was frequently dominant. Both the *Cortinarius* sp. and *L. laccata* infected fewer than half of the seedlings grown in their respective soils, but they were often the dominant types on those seedlings. The seedlings grown in all soil samples were also infected by a broad range of other mycorrhizal types. This was especially so in the '*Lactarius*', '*Cortinarius*' and '*Laccaria*' soils, and it is notable that the soils beneath the fruitbodies of these fungi also contained a large variety of mycorrhizal types originally. Nevertheless, several mycorrhizal types that occurred on the seedling roots were not observed in the soils themselves (Table 5.5.10).

The mean numbers of mycorrhizal tips on the seedlings are presented in Table 5.5.11. Lactarius-type mycorrhizas were the most numerous type overall, with Laccaria-type the second most common. These two types and type 3 mycorrhizas were numerically the dominant individual types on the seedlings grown in their respective soils. Leccinum-type and Amanita-type mycorrhizas accounted for a relatively small proportion of the root tips overall, and by far the majority of root tips were infected by the eleven mycorrhizal types listed in Table 5.5.10 and grouped together as 'others' in Table 5.5.11.

TABLE 5.5.11: Mean numbers (± s.e.) of root tips of different mycorrhizal types on seedlings grown for 141 days in a glasshouse in soil collected from beneath the fruitbodies of five species of fungi in Struan Wood.

Fruitbody beneath which soil was taken	Mycorrhizal types on seedlings						
	Amanita	*Leccinum*	*Lactarius*	*Cortinarius**	*Laccaria*	Others	Total
Amanita muscaria (n=11)	0	8 ± 5.0	11 ± 7.7	0	39 ± 15.0	23 ± 8.9	110 ± 15.3
Leccinum roseofracta (n=5)	0	0	16 ± 6.8	2 ± 2.4	0	35 ± 3.8	98 ± 10.6
Lactarius tabidus (n=20)	6 ± 5.9	4 ± 2.2	45 ± 9.6	0	0	20 ± 7.2	103 ± 13.4
Cortinarius sp. (n=17)	3 ± 3.3	2 ± 1.1	1 ± 1.0	15 ± 6.2	2 ± 1.9	35 ± 6.8	82 ± 10.5
Laccaria laccata (n=13)	0	1 ± 1.4	9 ± 4.3	3 ± 2.3	36 ± 17.3	48 ± 15.4	127 ± 17.1
Overall mean (n=66)	3 ± 2.0	3 ± 1.1	19 ± 3.9	4 ± 1.8	14 ± 4.6	31 ± 4.5	103 ± 6.6

* Type 3 mycorrhizas are assumed to be attributable to *Cortinarius* sp.

In some instances it appeared that the numbers of some mycorrhizal types showed an inverse relationship to the numbers of the 'fruitbody-type' mycorrhizas. For instance, in the '*Cortinarius*' soil only 5 mycorrhizal tips of type 17 were observed on the seven seedlings that bore Cortinarius-type (type 3) mycorrhizas whereas 65 tips of type 17 were observed on the remaining ten seedlings. This difference was statistically very highly significant (χ^2), even if it was analysed after the number of type 3 mycorrhizas was deducted from the total, because root tips infected by type 3 might be considered unavailable for infection by other types. A similar trend could also be observed on the '*Laccaria*' seedlings: the five seedlings with Laccaria-type mycorrhizas had only 4 mycorrhizal tips of *Inocybe* sp. whereas the remaining eight seedlings (without *Laccaria*) had 267 such mycorrhizal tips; this difference was, after correction, also very highly significant (χ^2).

The results for occurrence of mycorrhizas in the soils beneath different fungal fruitbodies closely resemble the results of Deacon *et al.* (1983) for a similar range of fungi; thus, the mycorrhizas of 'late-stage' fungi, such as *A. muscaria* and *L. roseofracta*, were dominant in the soils beneath their respective fruitbodies, whereas the mycorrhizas of 'early-stage' fungi, such as *L. laccata*, were not always present and were never dominant beneath the fruitbodies of these reported 'early-stage' fungi. Unlike the results reported by Deacon *et al.* (1983), considerable infection of the seedlings by 'late-stage' or assumed 'late-stage' fungi occurred; however, *A. muscaria* and *L. roseofracta* did not infect seedlings grown in the soil from beneath their respective fruitbodies. Also, *L. laccata*, an 'early-stage' fungus, was not always the dominant mycorrhizal fungus on seedlings grown in the soil from beneath its fruitbodies; this also contrasts with the work of Deacon *et al.* (1983). It thus seems from this investigation that an absolute distinction between 'early-stage' and 'late-stage' mycorrhizal fungi, based on the criterion of Deacon *et al.* (1983), is not possible in the soil from Struan Wood, though my results nevertheless show several similarities with that earlier work.

5.6 Discussion

The experiments in this section show that some mycorrhizal fungi that cannot establish infection on seedlings in isolated soil cores or in trenched areas of soil can nevertheless colonise seedlings planted around mature trees. There are several possible explanations of this behaviour, as discussed below, but the preferred explanation, consistent with most or all of the results, is that the inoculum of those fungi needs to remain attached to a food base - the parent tree - in order to establish mycorrhizas.

Lactarius pubescens showed a consistent pattern of behaviour in two experiments (Sections 5.2.1 and 5.3.1). When its inoculum was severed from the parent tree by either coring or trenching then it colonised only very few seedlings, and these only to a limited degree. In contrast, when the soil containing its inoculum was not 'isolated' then *L. pubescens* not only colonised the roots of many seedlings but also was over-represented on these root systems compared with on the parent tree root system. Such a result would not be expected if the existing mycorrhizas alone provided the inoculum source; rather, it seems likely that mycelial strands or extra-matrical hyphae from these mycorrhizas were responsible for mycorrhizal establishment on the seedlings. In this respect, the experiments show many parallels with those of Garrett (1954, 1956) and with his suggestion (Garrett, 1951) that mycelial strands and rhizomorphs of fungi are important as colonising organs, because Garrett (1954, 1956) found that attachment to an adequate food base is necessary for successful establishment at new sites from mycelial strands and rhizomorphs. Indeed, for a fungus like *Armillaria mellea* which needs such a food base, Garrett (1956) showed that the rate of growth and inoculum potential of the rhizomorphs are directly proportional to the volume of the food base. Such a demonstration could not be made in the experiments reported here, but it would be feasible in laboratory and glasshouse conditions by shading plants or otherwise reducing the amount of photosynthate available to mycelial strands.

It is notable that *L. pubescens* did not infect seedlings even if its inoculum was separated, on a large root volume, from the parent tree by trenching (Table 5.3.2). This indicates that the fungus cannot use saprophytically derived nutrients as a food base for infection nor can it use stored food reserves in the roots or in the mycorrhizas themselves. In this respect, the behaviour of *L. pubescens* differs from that of *Psalliota hortensis* Cke. (=*Agaricus hortensis* (Cke.) Pilat) and *Armillaria mellea* (Garrett, 1954, 1956), but such a difference is not surprising because the latter two fungi can grow saprophytically whereas *L. pubescens* probably depends almost exclusively on host-derived assimilates.

In the experiments in which *L. pubescens* did not infect seedlings because of coring or trenching of the soil, the seedling roots developed mycorrhizas attributable to reported 'early-stage' fungi such as *Hebeloma* sp., *Laccaria* sp. and *Inocybe* sp. The implication is that in the absence of infection by *L. pubescens* these fungi can colonise available seedling roots. The alternative explanation is that the experimental treatments had direct primary effects on these other fungi, which then excluded

L. pubescens, but this could not explain why *L. pubescens* was over-represented on seedlings in 'non-isolated' regions of soil, as mentioned above.

In stating these possibilities, it is recognised that cored and trenched areas of soil differ from non-isolated areas in several respects and that the potential importance of these differences remains largely unknown. For example, both water and mineral nutrient availability are likely to be greater in 'isolated' than in 'non-isolated' areas of soil beneath trees, and the difference in water availability is unlikely to have been nullified by the watering regime in my experiments because the mature trees would have created a substantial transpirational pull. Perhaps this provides the explanation of the enhanced growth of seedlings in isolated than in non-isolated areas of soil in some of the experiments (Section 5.2.1; Tables 5.3.1 and 5.3.5), though the seedlings showed no sign of drought stress. Gadgil and Gadgil (1971) found that litter decomposition was increased when tree roots were removed from areas of soil within a stand of *Pinus radiata*; similarly, there was an increase in soil moisture after small areas of forest soil were isolated from the surrounding trees, though there was no change in pH or nutrient status of the soil (Gadgil and Gadgil, 1975).

A problem with interpreting my results in terms of differences in water or nutrient supply is that there was no apparent effect of position of soil cores (see Figure 5.2.1) on the degree of mycorrhizal development (Section 5.2.1), whereas it would be expected that water and nutrient availability would vary in different zones around a tree. Also, it is difficult to explain on this basis why there was so little infection of seedling roots in non-isolated areas by fungi such as *Hebeloma* sp., *Laccaria* sp. and *Inocybe* sp. (Table 5.3.2), because mycorrhizal fungi have been shown, in general, to colonise seedlings more readily in soils of low than of high fertility (Marx *et al.*, 1982; Shaw *et al.*, 1982). On balance, therefore, the results of my experiments with *L. pubescens* are best explained by implicating mycelial strands (or extra-matrical hyphae) as infective units and by suggesting that these function most effectively when they have access to host-derived assimilates to enhance their inoculum potential (Garrett, 1970).

Some similarities between *Lactarius glyciosmus* and *L. pubescens* are apparent from studies on the experimental birch stand at Bush Estate. Both fungi, for example, appeared as fruitbodies around the trees only after 4 or more years (Last *et al.*, 1983). In the experiment described in Section 5.3.2, the colonisation of seedlings by *L. glyciosmus* showed a similar pattern to that observed for *L. pubescens*: in trenched and isolated positions there was significantly less infection by *L. glyciosmus* than in the non-isolated positions (Table 5.3.6). However, the difference between treatments was not as pronounced as for *L. pubescens*, possibly because *L. glyciosmus* occupied only a small proportion of the parent tree root system and the mycorrhizas of *L. glyciosmus* were not observed to produce mycelial strands. Indeed, unlike *L. pubescens*, *L. glyciosmus* was not over-represented on the seedling root systems compared with on the parent tree root system – a point that supports the view that *L. pubescens* infects seedlings from mycelial strands.

Leccinum spp., like *Lactarius pubescens*, can be considered 'late-stage' fungi according to the criterion of Deacon *et al.* (1983); that is, they do not colonise seedlings in soil cores or sieved soil from introduced or resident sources of inoculum. Further, fruitbodies of *Leccinum* spp. appear only later in the observed succession on birch trees (Mason *et al.*, 1983a). In the experiment in Section 5.3.2, it was shown that the numbers of Leccinum-type mycorrhizas on non-isolated seedlings were significantly greater than on seedlings grown in cores (Table 5.3.6) and in this respect the results closely parallel those obtained for *L. pubescens* and *L. glyciosmus* (Tables 5.2.1 and 5.3.2). All of these results thus demonstrate a consistent pattern of behaviour of proposed 'late-stage' fungi, represented by four species in two genera. Similar interpretations of the data could be made for infection by the *Leccinum* spp. (*L. scabrum* and *L. roseofracta*) as were made for *L. pubescens* - namely that these fungi depend on nutrients supplied from a food base (a mature tree) in order to colonise seedling roots in soil. But interpretation of the behaviour of *Leccinum* spp. was complicated by the fact that these fungi colonised some seedlings grown in trenched areas of soil, the degree of mycorrhizal development then being intermediate between that in non-isolated and in cored soil (Table 5.3.6). It can thus be suggested either that the trenching was ineffective in completely severing inoculum from contact with the parent tree or that *Leccinum* spp. can utilise nutrients in sufficiently large volumes of severed root in order to initiate infection of seedlings.

Of these suggestions, the first is preferred and it is supported by several observations made during the experiment. Most importantly, a fruitbody of *Leccinum roseofracta* was seen within one of the trenched areas, 29 days after trenching, and actively photosynthesising hosts are reported to be necessary for fruitbody production by most mycorrhizal fungi (Hacskaylo, 1965; Last *et al.*, 1979). Indeed, in the experiments of Rommell (1938) and Laiho (1970) the appearance of fruitbodies ceased when roots were isolated, by trenching, from parent trees. An additional point is that a proportion of the mycorrhizas present in the soil in the trenched areas were evidently alive (Table 5.3.7), whereas in the other trenching experiment (which showed the expected pattern of seedling infection) all the mycorrhizal tips remaining within the trenched areas appeared to be dead (c.f. Tables 5.3.3 and 5.3.7). Inocybe-type mycorrhizas were present in both experiments but were alive in one and dead in the other, so there is a clear basis for comparison between the experiments.

If, as suggested, there was incomplete isolation of trenched areas in the experiment involving *Leccinum* spp., then the question is raised as to whether the connection with the parent tree was by roots or mycelial strands. Laiho (1970) reported that the mycelial strands of *Paxillus involutus* proliferated in the immediate vicinity of a trench, where the soil was less compact, and grew underneath barriers inserted into trenches. However, the growth of mycelial strands of *Rhizopogon roseolus* - one of the few fungi investigated in this respect - has been reported to be 2-4 mm day^{-1} (Skinner and Bowen, 1972) and, even growing from the base of a trench, it would take 115 days for a mycelial strand growing at this rate to penetrate to the centre, at the surface, of one of the trenched areas in the present work. Indeed, the growth of mycelial strands is reported to be reduced where the soil is compact (Skinner and Bowen, 1974b), such as in subsoil at the base of a trench. It therefore seems unlikely that recolonisation by mycelial strands, alone, could have been responsible for the appearance of a fruitbody of *L. roseofracta* or for the numbers of mycorrhizas observed in trenched areas; rather, the preferred explanation is that not all of the roots in the trenched areas were completely severed.

The second possible explanation for the results obtained with *Leccinum* spp., namely that a large isolated root volume can provide a food base for the colonisation of seedlings, can be supported by only little evidence. Dimbleby (1953) suggested that the inoculum of *Boletus scaber* Fr. (*Leccinum scabrum*) and *Amanita muscaria* may have survived on old pine stumps such that they were able to colonise naturally regenerating birch trees growing around the stumps. However, the birch trees that he examined were at least 8 years old, an age by which infection by *Leccinum* spp. may be expected in any case (Mason *et al.*, 1983). Harvey, Jurgensen and Larsen (1980) reported the survival of 'active' mycorrhizal tips on conifer stumps up to 9 months after clear-felling. However, their assessment of active mycorrhizal tips was based on only the visual appearance of the mycorrhizas and these workers made no attempt to determine if these root tips would function as inoculum for the colonisation of seedling roots. It should be noted, nevertheless, that in the experiment with *Leccinum* spp. all of the trenched areas were remarkably similar, one to another, with regard to both the colonisation of the seedlings by different mycorrhizal fungi and the occurrence of different mycorrhizal types on parent tree roots in sampled soil cores. Such consistency seems incompatible with the suggestion that trenched areas were incompletely isolated and is, rather, more compatible with the view that residual root volumes provided inoculum bases for infection by *Leccinum* spp. In short, it is impossible to be certain whether or not *Leccinum* spp. can establish mycorrhizas from detached food bases, but it is notable that, in the same experiment, *Lactarius glyciosmus* evidently could not do so.

In one more experiment in this series involving isolation of soil inoculum, an attempt was made to study the behaviour of *Tricholoma fulvum* (Section 5.2.2). It was thought that this fungus would behave similarly to the others in many respects, because it produces abundant mycelial strands and its fruitbodies have not been observed around young birch trees (P.A. Mason - personal communication) or on seedlings grown in soil in a glasshouse. However, *T. fulvum* colonised very few seedlings in the experiment (Table 5.2.3), and its failure to do so could not be attributed to isolation from the host or to a lack of suitable inoculum in the soil. The reason why *T. fulvum* did not colonise seedling roots remains unclear, but it is possible that this represents a further type of behaviour, distinct from that of the 'early-stage' and 'late-stage' fungi examined so far.

It must be stressed that although distinct patterns of fungal behaviour were observed in the experiments in this section, yet the work was done in only one site. For this reason, it was thought necessary to examine seedlings in more natural woodland environments (Section 5.5).

Seedlings growing around chestnut coppice stools bore a predominance of a strand-forming mycorrhizal type that did not colonise seedlings grown in the same soil in a glasshouse. This clearly parallels the experimental evidence discussed above, insofar as a mycorrhizal type that was present on seedlings growing in the root zone of mature trees did not occur on seedlings in isolated volumes of soil. It should be noted that although the coppice stools had been cut, they were not dead; all of the stumps had produced a number of vigorous shoots and so photosynthate would have been available to the roots. However, notable differences in the growth of seedlings occurred in the glasshouse compared with in the field so, clearly, the factors that limited seedling growth in field conditions did not do so in the glasshouse. This, together with the fact that many of the mycorrhizal types that occurred on seedlings in the glasshouse were not observed in the field, suggests the need for caution in interpreting the results. Field experiments involving isolation treatments, such as those done in the experimental birch plot at Bush Estate, would have been more satisfactory for the study of mycorrhizal colonisation on naturally regenerating seedlings.

There were also problems of interpretation with the three experiments that involved transplanting of seedlings to or from field sites. The persistence of established mycorrhizas on birch seedlings after transplanting to the chestnut coppice (Table 5.5.7) could not be determined because the majority of seedlings died, probably from drought; their counterparts grown in the same soil in a glasshouse did not provide a valid comparison because of the differences in growth between the glasshouse-grown and the surviving field-grown plants. Similarly, the rapid growth of naturally regenerated seedlings when collected from the coppice and transferred to a glasshouse (Table 5.5.4 and 5.5.5) may have been a major factor in the disappearance from the root systems of some of the formerly established mycorrhizal types and also for the colonisation of the roots by mycorrhizal types common in the glasshouse but not observed on seedlings in the field.

The results from the investigation at Struan Wood provide some contrasts with other work reported here. Whereas the occurrence of different types of mycorrhizas in soil beneath the fruitbodies of the different fungi was as expected from previous work by Deacon *et al*. (1983), yet the occurrence of mycorrhizas on seedlings grown in these soil samples was not as reported by these workers. Mycorrhizas of *Lactarius tabidus* were dominant on the seedlings (Table 5.5.11), even though the soil inoculum was 'isolated' from a mature host. This behaviour is different from that obtained for two other species of *Lactarius*, namely *L. pubescens* and *L. glyciosmus*, in work based on the experimental birch plot on the Bush Estate (Deacon *et al.*, 1983; see also Sections 5.2.1, 5.3.1 and 5.3.2). Indeed, the degree of colonisation of the seedlings by *L. tabidus* was such as would be expected for seedlings growing in 'non-isolated' positions around mature trees. There was also a surprising degree of infection by the two reported 'late-stage' mycorrhizal fungi, *Leccinum roseofracta* and *Amanita muscaria* when seedlings were grown in the glasshouse in soil from Struan Wood; although, overall, there were few mycorrhizas of these fungi on the seedling roots in glasshouse conditions, yet even this degree of development would not be expected from the results of other work (Deacon *et al.*, 1983; Sections 4.2.1 and 5.3.2). Interestingly, there was no infection of seedlings by these fungi in the soil samples in which their inoculum was most abundant, i.e. from beneath their fruitbodies (Table 5.5.10); rather, these fungi established mycorrhizas in some of the other soil samples. It is reported that the mycelial nutrient reserves of some fungi are severely depleted by the production of fruitbodies. For example, Madelin (1956) demonstrated that fruitbody production by *Coprinus lagopus* Fr. involved the withdrawal of nutrients from a whole colony. Also, Wessels and Sietsma (1979) showed that the production of fruitbodies of *Schizophyllum commune* Fr. entails extensive utilisation of mycelial nutrient reserves, including the products of hyphal wall autolysis. So from these studies on other basidiomycetes, and especially from the extremely detailed work of Wessels and Sietsma (1979), it seems likely that at least some mycorrhizal fungi, like *Leccinum* sp and *A. muscaria*, might deplete the mycelial nutrient reserves immediately below the fruitbodies during fruiting, such that the inoculum remaining in the soil, when isolated from a parent tree, has insufficient nutrients to establish new infections.

Even the behaviour of *Laccaria laccata*, the only known 'early-stage' fungus studied from Struan Wood, was atypical of that reported by Deacon *et al.* (1983) from studies on the Bush Estate. *L. laccata* colonised less than half of the seedlings grown in soil from beneath its fruitbodies, whereas Deacon *et al.* (1983) reported that *L. laccata* always colonised seedlings grown in soil from beneath its fruitbodies, even when Laccaria-type mycorrhizas were not observed in soil cores. The results from Struan Wood thus differ from those of the rest of the work in this chapter in three major respects. Firstly, an 'early-stage' fungus, *L. laccata*, did not always colonise seedling roots in soil from beneath its fruitbodies; secondly, there was at least some degree of infection of seedlings by the two 'late-stage' fungi, *A. muscaria* and *L. roseofracta*; thirdly, there was extensive colonisation of seedlings in 'isolated' soil by a *Lactarius* species, *L. tabidus*. The reasons for such differences are not known and require further study, but a possible explanation is that different soil conditions or related differences in the soil microflora may have affected the abilities of some fungi to colonise seedling roots. It is notable, in this respect, that unlike the soil at Bush Estate or in the chestnut coppice, there was a deep humus and litter layer at Struan Wood and this comprised at least part of each composite soil sample in which the seedlings were grown in the glass-house. Work described in Section 4.3.2 showed clearly a difference in behaviour of *Hebeloma sacchariolens* in different soils, and similar soil effects have been reported by Mason *et al.* (1983a). The concept of 'early-stage' versus 'late-stage' mycorrhizal fungi, and even the concept of mycorrhizal succession in general, may therefore have to be viewed in relation to soil and other abiotic and biotic variables.

SECTION VI

Concluding Discussion

Evidence for a succession of mycorrhizal fungi on birch came originally from observations on the appearance of fruitbodies of such fungi around birch trees as they aged (Mason *et al*., 1982). Deacon *et al*. (1983) demonstrated that this was also a succession of the mycorrhizas themselves. The succession was both temporal, because more fungi appeared as the trees aged (Mason *et al*., 1982), and spatial, because mycorrhizas and their fruitbodies occupied distinct zones around the trees (Ford *et al*., 1980; Deacon *et al*., 1983). Furthermore, Deacon *et al*. (1983) showed that two groups of mycorrhizal fungi, termed 'early-stage' and 'late-stage', could be distinguished; 'early-stage' fungi could colonise seedling roots from resident or introduced inoculum in samples of soil whereas 'late-stage' types could not do so. My experiments provide additional evidence for a mycorrhizal succession on birch and establish further differences in behaviour between 'early-stage' and 'late-stage' mycorrhizal fungi (*sensu* Deacon *et al*., 1983), supporting the separation of these broad categories.

The experiment described in Section 4.6 showed how spatial sequences of mycorrhizas, such as those described by Deacon *et al*. (1983), could develop. The mycorrhizas of *Lactarius pubescens* and an unknown fungus were found predominantly on roots close to the stem base, i.e. in the older part of the root system, whereas the younger distal root regions were colonised mainly by *Inocybe* sp. Similarly, this and another experiment (Section 4.2.1) demonstrated a clear temporal sequence in the development of the mycorrhizas of *L. pubescens*. Although not initially present on the seedlings after propagation, this fungus colonised an increasing number of seedlings and a greater proportion of root tips as the seedlings aged; the incidence of infection by *L. pubescens* was highest after the seedlings had undergone dormancy. This work provides clear evidence of a 'true' fungal succession on the roots of birch and satisfies the criterion proposed by Hudson (1968) for a fungal succession, namely that different fungi arrive and colonise at different times. However, parallels can be made with the coprophilous fungal succession analysed by Harper and Webster (1964) and shown to be largely a succession of fruiting rather than one involving sequential phases of growth, because although *L. pubescens* was present on some seedling roots within a year of seed germination no fruitbodies of this fungus appeared until the saplings' fourth growing season; it must therefore be recognised that mycorrhizas may form long before they are able to produce fruiting structures.

The distinction of mycorrhizal fungi into 'early-stage' and 'late-stage' types (*sensu* Deacon *et al.*, 1983) is supported by my experiments. 'Late-stage' fungi, such as *L. pubescens*, *Leccinum* sp. and *Amanita muscaria*, did not colonise seedlings that were grown either in soil cores taken from beneath fruitbodies or in soil supplemented with vermiculite-peat inoculum of these fungi (Sections 4.2 and 4.5.2); rather, the seedlings were colonised by naturally occurring fungi such as *Hebeloma* sp. and *Thelephora terrestris*, i.e. typical 'early-stage' fungi (*sensu* Deacon *et al.*, 1983). Similarly, Fox (1983) has reported that *Hebeloma* spp. and *Inocybe* spp. readily colonised seedling roots from basidiospore inocula whereas *L. pubescens* and *Leccinum roseofracta* did not do so. This distinction of mycorrhizal fungi into two categories seems to be unequivocal insofar as it relates to the establishment of infection from 'isolated' or dispersed fungal propagules in soil. Generally, it seems that this distinction applies also to the spread of infection from artificially established mycorrhizas when seedlings are transplanted to unsterile soil;

'early-stage' types colonise new roots readily whereas 'late-stage' types do not do so (Section 4). It thus seems that factors that influence the establishment of infection on the root system also limit the progress of infection from established mycorrhizas. Despite these comments, my work suggests that the 'late-stage' category as recognised by Deacon *et al.* (1983) may include a range of fungi with differing patterns of behaviour. For example, *Leccinum* sp. and *A. muscaria* never colonised seedling roots in soil from added inoculum or from artificially established mycorrhizas (Section 4) and in this respect it has been demonstrated that *A. muscaria* does not colonise the roots of Douglas-fir until the trees are over 12 years old (Chu-Chou and Grace, 1981). By contrast, *L. pubescens* colonised seedlings within a year in my experiments and also spread, albeit poorly, from established mycorrhizas (Section 4). In addition to these points, it must be recognised that the concept of mycorrhizal succession is based at present on only one or a few soil types and it should be considered in relation to other biotic and abiotic factors that may affect the formation of mycorrhizas. For example, soil type was demonstrated to have a marked effect on the ability of *Hebeloma sacchariolens* and *Paxillus involutus* to colonise seedling roots (Sections 3.3.1 and 4.3.2).

Of especial interest, my experiments have demonstrated that the 'late-stage' mycorrhizal fungi, *L. pubescens* and *Leccinum* spp., readily colonise seedlings in soil providing that their inoculum is not severed from a mature parent tree (Section 5). The evidence strongly suggests that these fungi rely on mycelial strands to initiate infection. Mycelial strands are by no means an exclusive feature of 'late-stage' mycorrhizal fungi but 'late-stage' fungi seem to differ from other strand-formers, such as *Thelephora terrestris*, in that they rely on host-derived assimilates in order to have sufficient inoculum potential (*sensu* Garrett, 1970) to colonise seedling roots. It also seems that 'late-stage' fungi colonising seedling roots from a food base provided by a mature tree have a competitive advantage over other mycorrhizal fungi because they colonised a greater proportion of seedling roots than their numbers on the parent tree root system would suggest.

This has important implications for the sequences of mycorrhizal fungi that may develop on naturally regenerating tree seedlings or on outplanted nursery stock. Seedlings growing on formerly tree-less sites are likely to be infected initially only by 'early-stage' mycorrhizal fungi, such as *Hebeloma* spp. and *Laccaria* spp.; interestingly, these fungi are referred to as 'pioneer' fungi by Watling (1981) because of their association with saplings invading open land. Ultimately, the trees are colonised by a greater range of fungi including 'late-stage' types as defined earlier; such a situation directly parallels the succession observed on the experimental birch plot on the Bush Estate (Mason *et al*., 1982) and is a clear case of a primary succession. By contrast, seedlings regenerating within the root zone of living mature trees may bear, from the outset, fungi that have until now been considered 'late-stage' (*sensu* Deacon *et al*., 1983). The species composition of mycorrhizal fungi in old, established woodland may thus remain relatively constant and thus represent a 'climax' community (Frankland, 1981).

It is not clear what effect a major disturbance, such as clear-felling, may have on subsequent sequences of mycorrhizal development. Frankland (1981) reported that fungi that were late in colonising established sand dunes were more sensitive to disturbances, such as blow outs, than were the typical earlier colonisers. My work suggests that following coppicing of broad-leaved trees, where the stumps remain alive and sprout new shoots, 'late-stage' fungi may persist and colonise naturally regenerating seedlings growing around the stumps (Section 5). However, where the stumps do not coppice, which is the case with many conifers, like pine and spruce and so no photosynthate is available to the roots, it is possible that a secondary succession may develop, beginning, again, with the 'early-stage' pioneer fungi.

It now seems that the terminology used to describe the two groups of fungi, 'early-stage' and 'late-stage' (*sensu* Deacon *et al.*, 1983) may be inappropriate, because 'late-stage' fungi may colonise seedlings growing around mature trees. However, the distinction between the two groups is quite clear and has provided, to date, a convenient framework within which to investigate the behaviour of mycorrhizal fungi. Moreover, it seems that similar successions, based on the appearance of fruitbodies or on results from root isolations, occur on trees other than birch, namely *Pinus radiata* (Chu-Chou, 1979), *Pseudotsuga menziesii* (Chu-Chou and Grace, 1981) and *Eucalyptus* sp. (Chu-Chou and Grace, 1982). Future investigation could usefully be focussed on the reasons for mycorrhizal successions. In the case of higher plant successions there are examples in which early colonists, by their activities, modify the habitat such that later colonists can invade. It is not easy to envisage such a mechanism for mycorrhizal successions on the basis of present information. Indeed, in an experiment designed specifically to test the possibility there was no evidence of a 'priming' effect of established 'early-stage' mycorrhizal fungi in enabling 'late-stage' ones to colonise seedlings (Section 4.2.1). Frankland (1981) suggested that the fungal succession observed in sand dunes with time was due to the habitat becoming more complex, with the development of soil profiles and accumulation of organic matter, thus providing new niches in which fungi may become established. Likewise, the establishment of a greater range of mycorrhizal fungi may be influenced by the increased diversity of environmental conditions around trees as they age, when soil conditions (Miles, 1977), root exudates (Smith, 1970) and soil microflora all may vary. The development of a humus layer is of especial interest because in a soil with a mor humus accumulation from an old birch woodland 'late-stage' fungi were able to colonise seedlings in glasshouse conditions. By contrast, there was no noticeable accumulation of organic matter in either the experimental birch stand at Bush Estate or the chestnut coppice at Rochester, Kent and in these cases the 'isolated' volumes of soil did not enable 'late-stage' mycorrhizal fungi to develop from resident or introduced inoculum. Clearly the factors, such as soil type, that affect the establishment of 'late-stage' fungi require further investigation. Until the factors responsible for the succession are determined it would seem premature to suggest alternative terminology for 'early-stage' and 'late-stage' mycorrhizal fungi - any such terminology could not, as yet, be based on necessarily the most appropriate biological criteria.

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APPENDIX 1

Descriptions of Mycorrhizal Types on the Roots of Birch

PLATE 4: Mycorrhizas of *Paxillus involutus*.

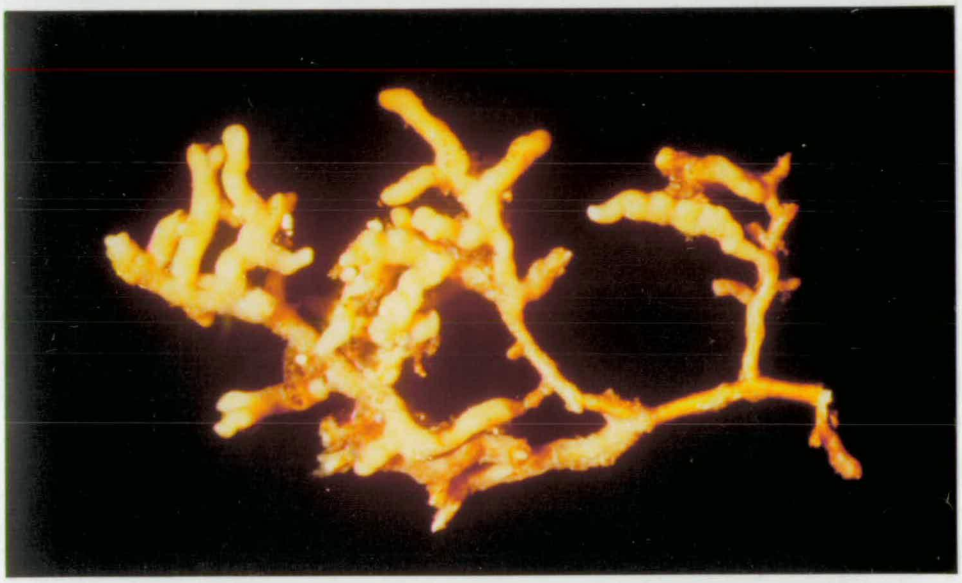
PLATE 5: Mycorrhizas of *Hebeloma sacchariolens*.

PLATE 6: Mycorrhizas of *Leccinum* sp.

PLATE 7: Mycorrhizas of *Thelephora terrestris*.

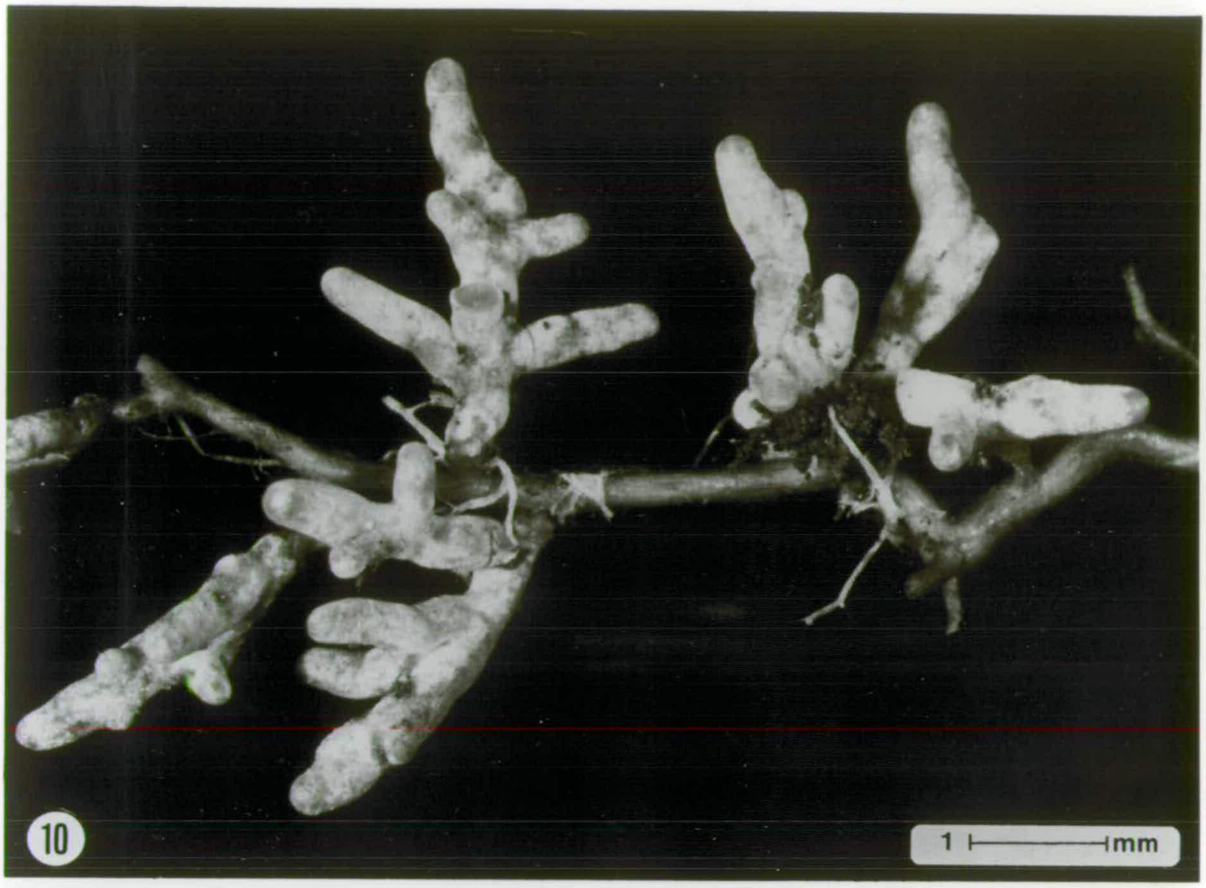
PLATE 8: Mycorrhizas of *Elaphomyces granulatus*.

PLATE 9: Type 2 mycorrhizas, brown with cream tips.

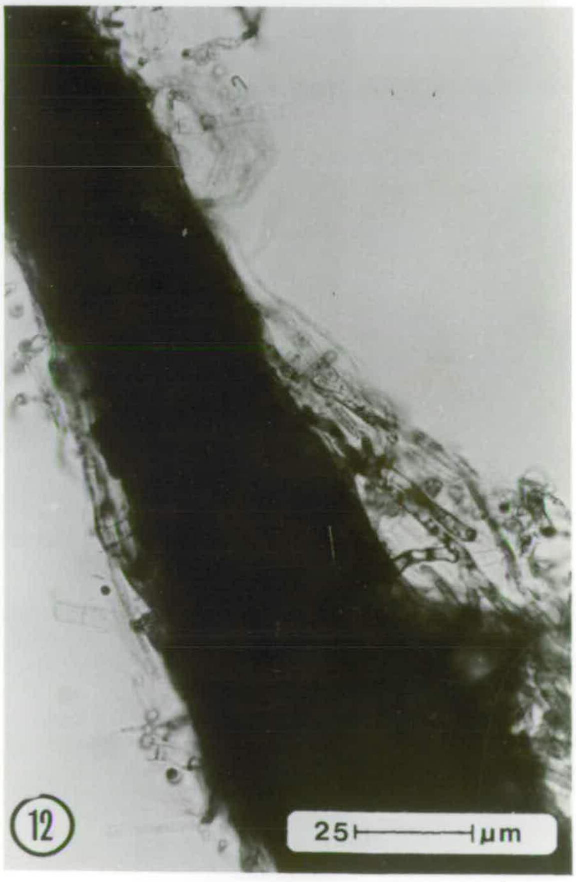


Amanita muscaria (L. ex Fr.) Pers. ex Hooker. Plates 1, 10, 11, 12.

Basis of identification	Pure culture synthesis; association with fruitbodies.
Gross morphology	Short (<3.5 mm long), stubby (up to 0.45 mm wide), multiple branched with hemispherical tips; little or no evidence of tapering at branch points or at origin from root axis (Plates 1 and 10).
Sheath	25-30 µm thick, surface silvery (Plate 1) going fawn with age or on bruising; sheath has distinctive appearance when examined by scanning electron microscopy (Plate 11).
Extramatrical mycelium	Sparse, generally in angles between mycorrhizal branches, composed of short hyphae (20-30 µm long and 2-3 µm wide).
Mycelial strands/ sclerotia	No sclerotia observed; mycelial strands present, white, generally short, up to 1.2 mm long and 60 µm wide (Plate 12).


10
1 mm


11
10 µm


12
25 µm

Tricholoma fulvum (DC. ex Fr.) Sacc.   Plates 13, 14, 15.

Basis of identification	Pure culture synthesis; association with fruitbodies.
Gross morphology	Individual elements tortuous, up to 0.4 mm diameter with frequent and multiple branches (Plate 13). Young mycorrhizas have an open ramiform branching pattern; branches form dense, tightly packed clusters with age.
Sheath	Surface silvery white but may turn pale fawn to black with age or on bruising; surface is a loosely organised network of hyphae (Plate 14); sheath 10-16 µm thick; Hartig net extends 2-3 cortical cell layers.
Extramatrical mycelium	Branched hyphae, 2-3 µm wide, extending up to 100 µm from the sheath surface; the hyphae seem to have granular cytoplasm; no clamps seen.
Mycelial strands/ sclerotia	Conspicuous mass of mycelial strands* (Plate 13) obscuring individual elements of the mycorrhiza. Strands coloured like the sheath surface, smooth and regular in surface appearance (Plate 15); up to 0.2 mm diameter and more than 1 cm long with frequent and regular branching. No sclerotia observed.

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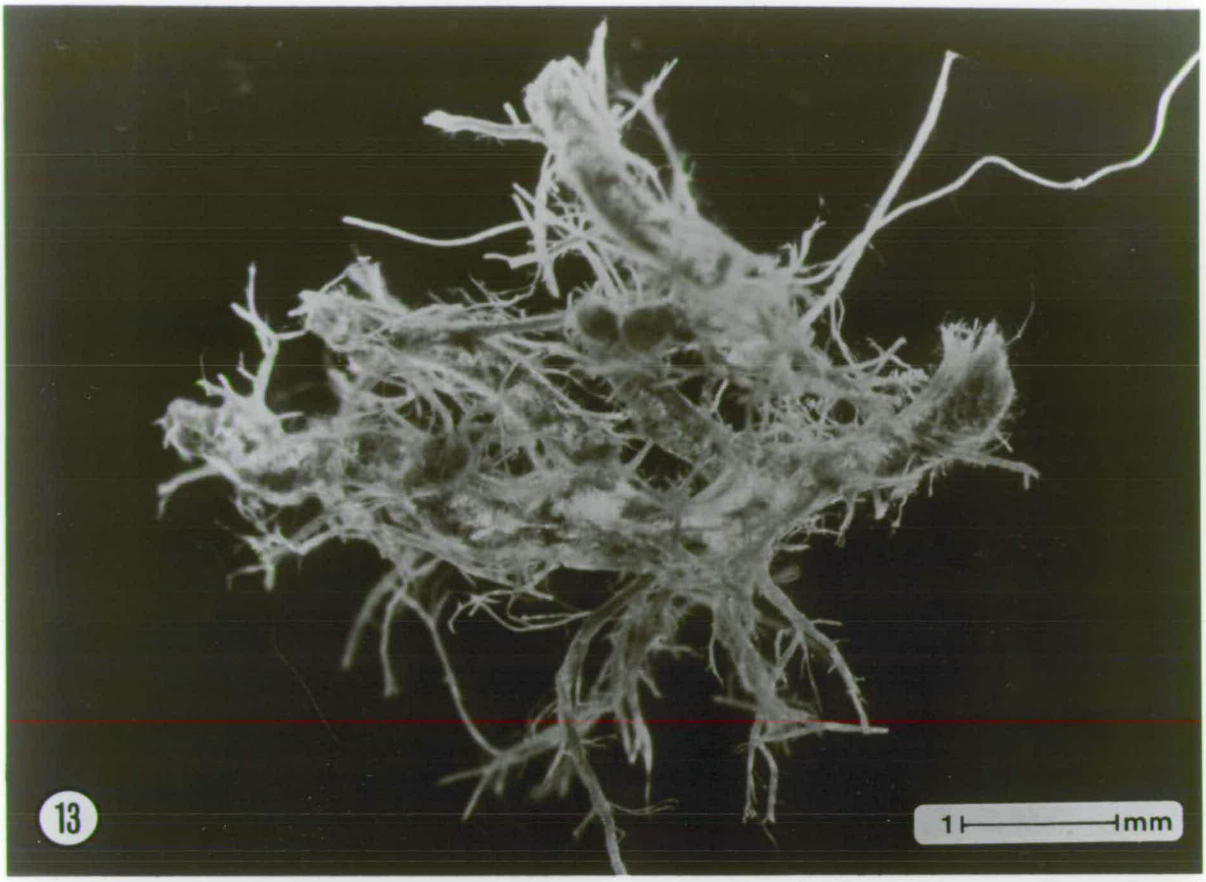
*Prominent features of different mycorrhizal types are underlined.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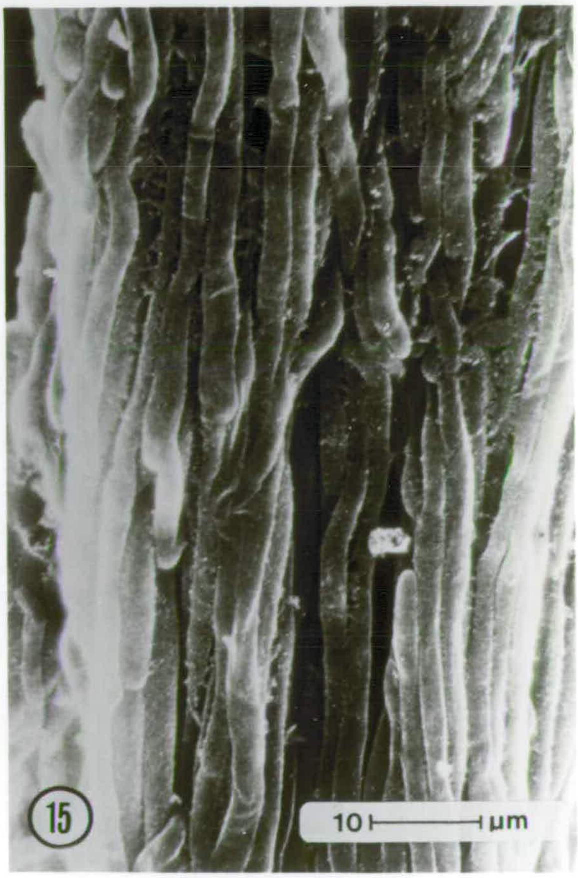
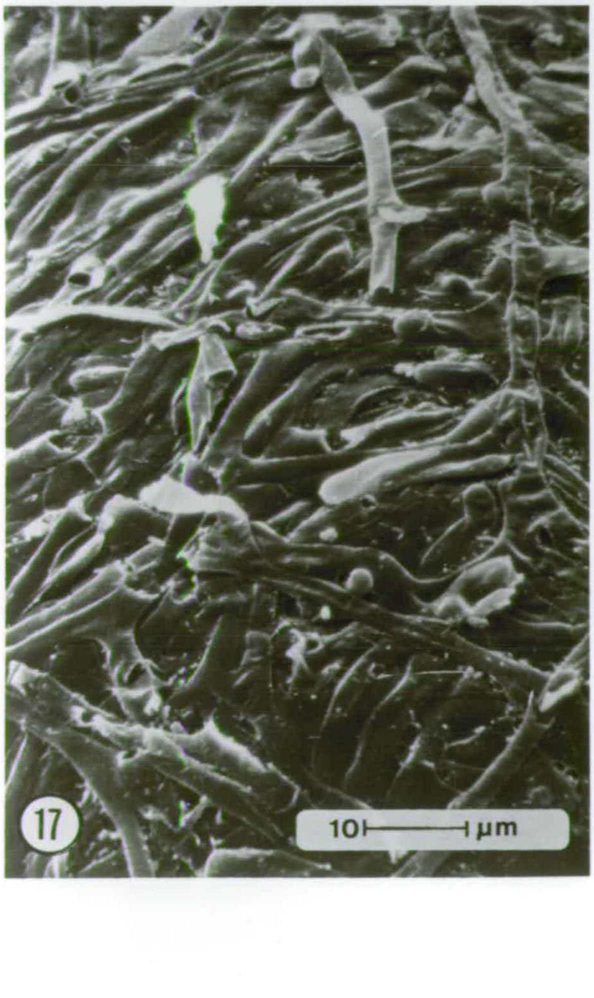
14


15

Laccaria spp. Plates 16, 17, 18.

Basis of identification	Pure culture synthesis; association with fruitbodies; seedling assay of inoculum-supplemented soil.
Gross morphology	Long, slender (0.3 mm wide) mycorrhizas with frequent but widely spaced branches (Plate 16); individual elements are regular and cylindrical but taper towards the apex and also, to a lesser extent, towards the base.
Sheath	Surface white; thin (7.0 - 8.8 µm thick); the outline of the cortical cells and of the stele are frequently visible through the root; sheath surface smooth with dense covering of flattened hyphal network (Plate 17).
Extramatrical mycelium	Generally sparse; where present, the hyphae tend to be appressed but may radiate out to 100 µm from the sheath; clamps, with holes, are frequent (Plate 18).
Mycelial strands/ sclerotia	None observed.
Notes	It was not possible to distinguish between the mycorrhizas of different *Laccaria* species.


16


17


18

Lactarius pubescens (Fr. ex Krombh.) Fr.   Plates 2, 19, 20, 21.

Basis of identification	Pure culture synthesis; association with fruitbodies.
Gross morphology	Individual elements slightly tortuous, generally of uniform diameter (0.3 - 0.4 mm wide); branches frequent but widely spaced, resulting in open branched systems which may be regularly doubly-pinnate (Plates 2 and 19).
Sheath	Orange (Plate 2), smooth with a distinct 'jigsaw' surface appearance when examined by light or scanning electron microscopy (Plate 21); sheath 7.6 - 11.4 μm thick with a well-developed Hartig net penetrating 3 cortical cell layers deep.
Extramatrical mycelium	Uncommon.
Mycelial strands/ sclerotia	Frequent mycelial strands concolorous with the sheath, up to 40 μm wide and smooth and regular in surface appearance (Plate 21); the strands are frequently observed to grow ectotrophically along woody birch roots. No clamp connections or sclerotia observed.


19


20


21

Lactarius glyciosmus (Fr. ex Fr.) Fr. Plates 22, 23.

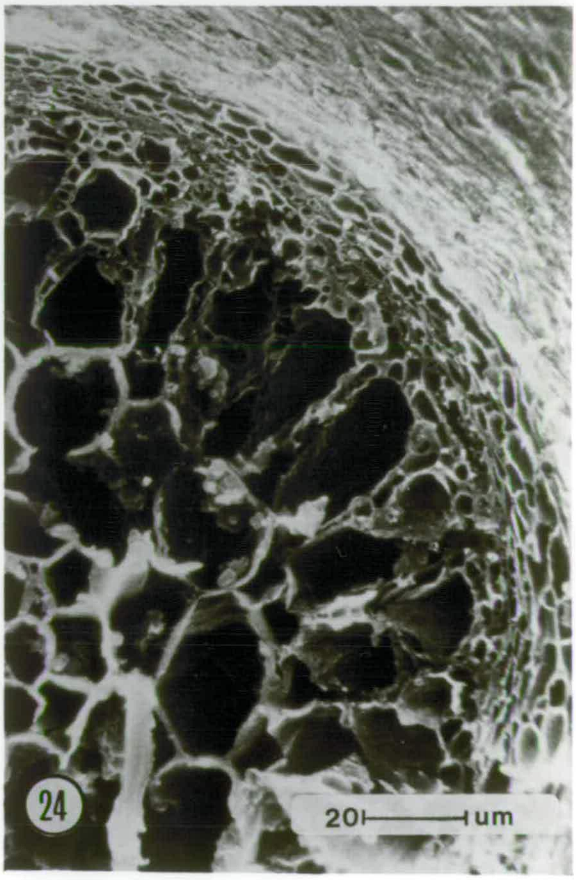
Basis of identification	Association with fruitbodies; seedling assay.
Gross morphology	Short, blunt mycorrhizas, 0.3 - 0.4 mm wide; individual elements smooth and uniform in diameter (Plate 22), occasionally branching. All the mycorrhizas observed of this fungus have appeared to be young, so branching patterns may become more apparent with age.
Sheath	Greyish to orange, surface very smooth (Plate 23) with no 'jigsaw' appearance; sheath 18.0 µm thick; well developed Hartig net.
Extramatrical mycelium	None observed.
Mycelial strands/ sclerotia	None observed.

Lactarius tabidus Fr. Plates 3, 24.

Basis of identification	Association with fruitbodies; seedling assay.
Gross morphology	Uniform thick mycorrhizas, up to 0.6 mm wide, individual elements slightly tortuous, up to 6 mm long. Branch frequent, regular (Plate 3) giving doubly pinnate appearance.
Sheath	Yellow to yellow-orange, glossy, darkening with age, 21-23 µm thick (Plate 24); surface smooth and, unlike *Lactarius pubescens*, without 'jigsaw' appearance.
Extramatrical mycelium	None observed.
Mycelial strands/ sclerotia	None observed.

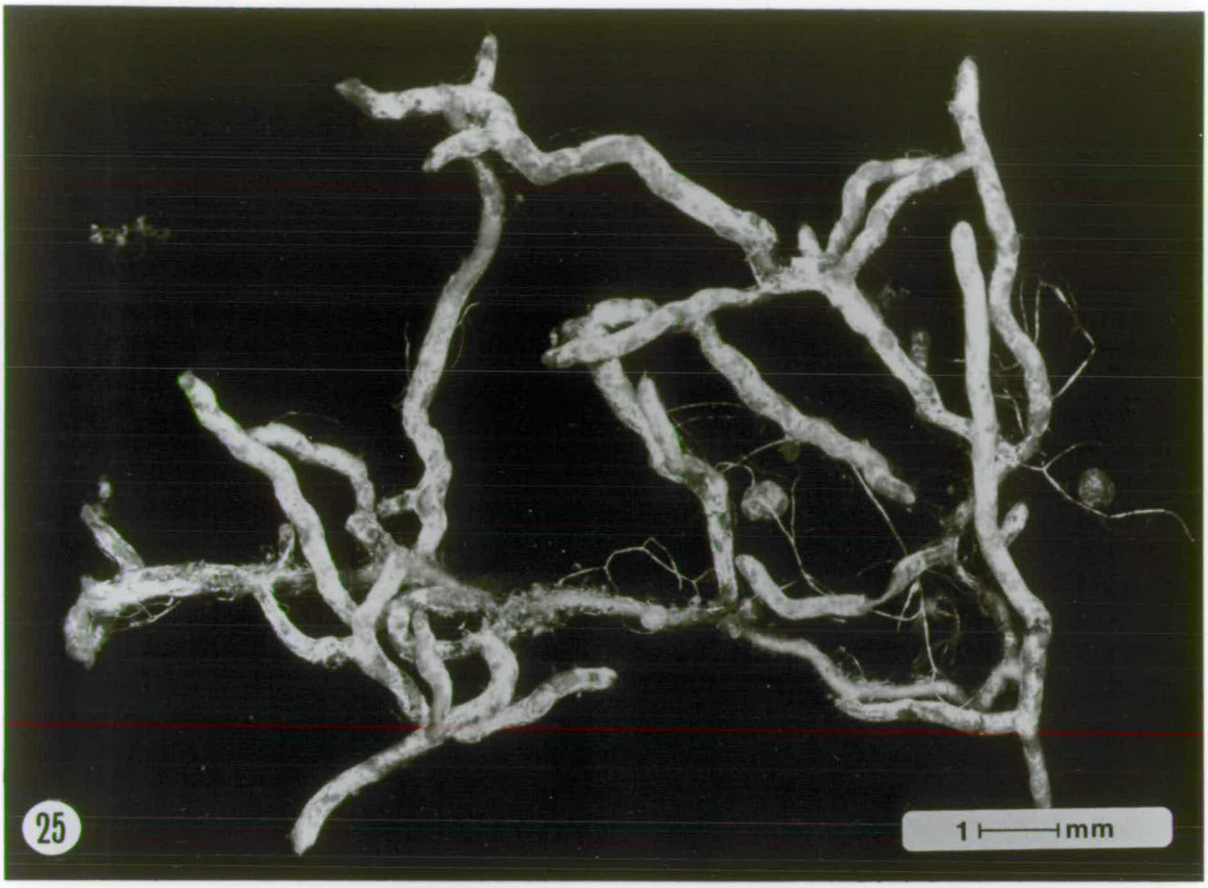

22


23


24

Paxillus involutus (Batsch.) Fr. Plates 4, 25, 26, 27.

Basis of identification	Pure culture synthesis; association with fruitbodies; seedling assay of inoculum-supplemented soil.
Gross morphology	Mycorrhizas sinuous with frequent but irregularly spaced branches; individual elements 0.2 - 0.4 mm wide and up to 8 mm long (Plate 25).
Sheath	Distinctive tawny yellow/golden brown in colour (Plate 4); surface loosely organised network of hyphae; sheath thickness 13-18 µm. Hyphae grow ectotrophically along the root surface, often in advance of formation of a true sheath.
Extramatrical mycelium	Branched hyphae, 3 - 4.5 µm wide and with numerous clamp connections (Plate 27), extending to 120 µm from the sheath surface.
Mycelial strands/ sclerotia	Mycelial strands concolorous with the sheath, abundant, up to 60 µm wide and surrounded by a fringe of hyphae (Plate 26). Globose sclerotia are borne on the mycelial strands; they vary from 0.28 - 0.8 mm diameter and are the same colour as the sheath.



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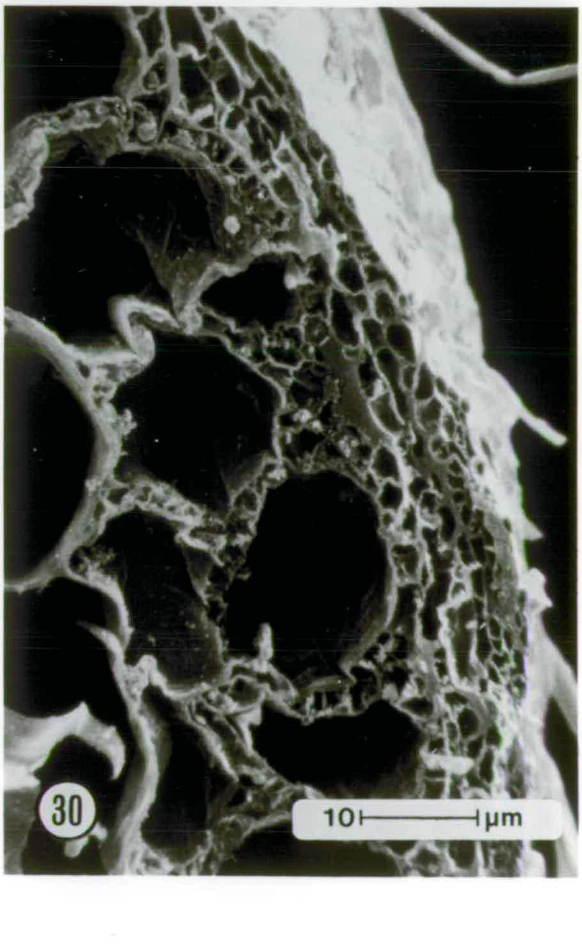
Hebeloma spp.

Plates 5, 28, 29, 30.

Basis of identification	Pure culture synthesis; association with fruitbodies; seedling assay of inoculum-supplemented soils.
Gross morphology	Individual elements may be up to 1 cm long (Plate 28) but are generally shorter, they may bear several irregularly spaced branches; elements are generally of uniform diameter (0.25 - 0.3 mm) and straight to tortuous.
Sheath	White with silvery patches, surface smooth but frequently obscured by surrounding mycelium, 9.5 - 14.0 µm thick (Plate 30).
Extramatrical hyphae	Dense, sometimes extending over 1 mm from the mycorrhiza, white, composed of frequently branched hyphae with abundant clamp connections (Plate 29).
Mycelial strands/ sclerotia	No mycelial strands observed. Sclerotium-like bodies present on mycorrhizas of *H. sacchariolens* Quélet (Plate 5). They are white, globose, up to 0.5 mm diameter, closely associated with, and attached to, the mycorrhizal surface.
Notes	The mycorrhizas of *H. sacchariolens* are similar in every respect to the mycorrhizas of other *Hebeloma* spp. but they are immediately distinguished by the sclerotium-like bodies described above.


28
1 mm


29
10 µm

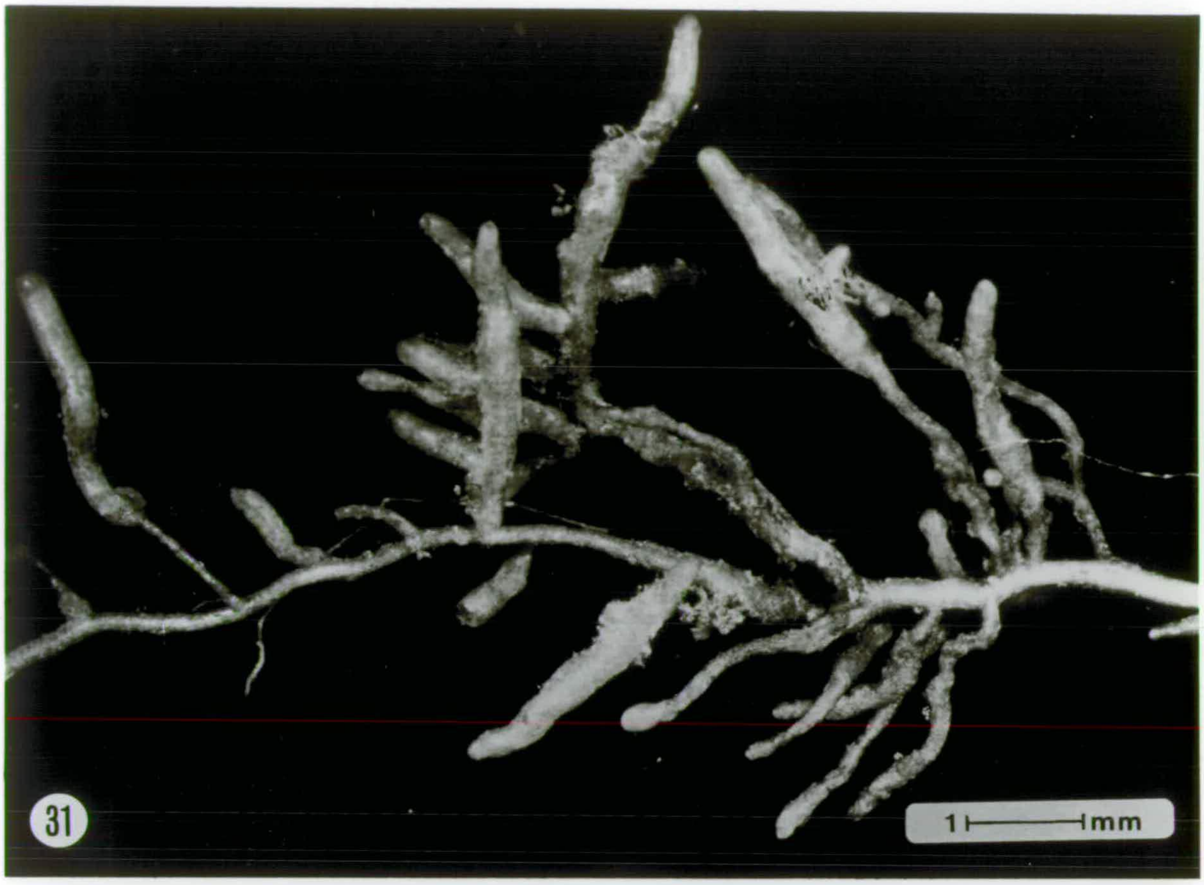

30
10 µm

nocybe lanuginella (Schroet) Konrad. & Maubh. Plates 31, 32.

Basis of dentification	Association with fruitbodies; seedling assay of spore-supplemented soils.
Gross morphology	Individual elements irregularly, swollen (Plate 31), up to 0.3 mm wide, often with a 'beaded' appearance; elements generally tortuous but occasionally straight, branches frequent, irregularly spaced.
Sheath	Surface smooth, off-white to grey in colour, outer cortical cells often visible as mottled patchwork beneath sheath surface; sheath 16-20 µm thick.
Extramatrical mycelium	Dense in patches, irregularly spaced over the mycorrhiza and associated with adhering soil particles. Hyphae narrow (1.6 µm wide), extending to 250 µm from the sheath, with frequent clamps that have prominent holes (Plate 32).
Mycelial strands/ sclerotia	None observed.

Inocybe lacera (Fr.) Quelet. Plate 33.

The mycorrhizas of this *Inocybe* sp. differ from *I. lanuginella* in having a generally smoother appearance, broader hyphae with no holes apparent in the clamp connections, and a slightly thinner sheath (12-16 µm thick, Plate 33).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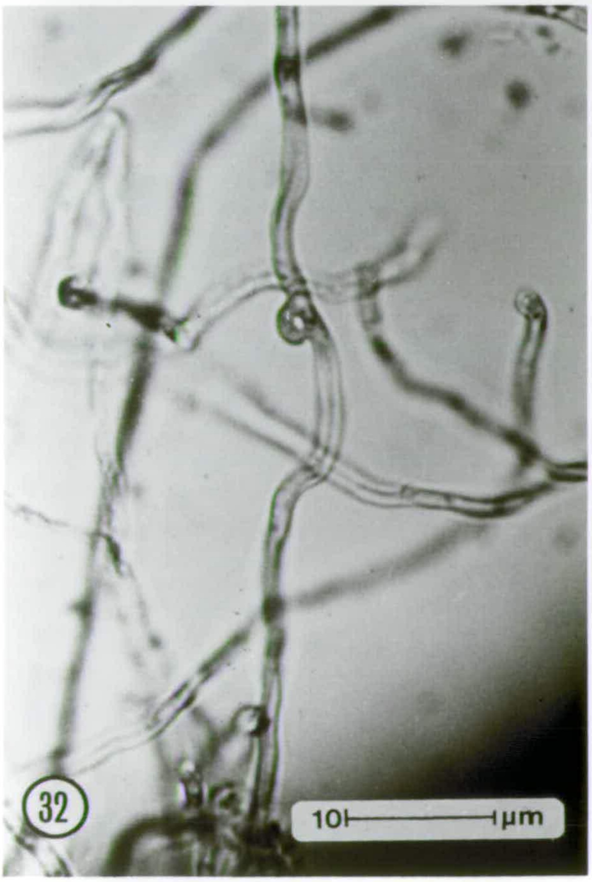
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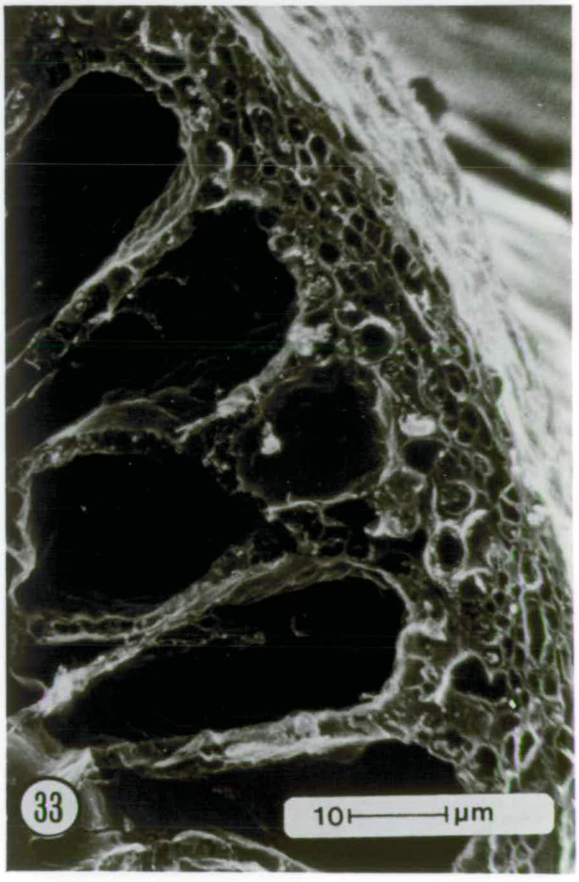
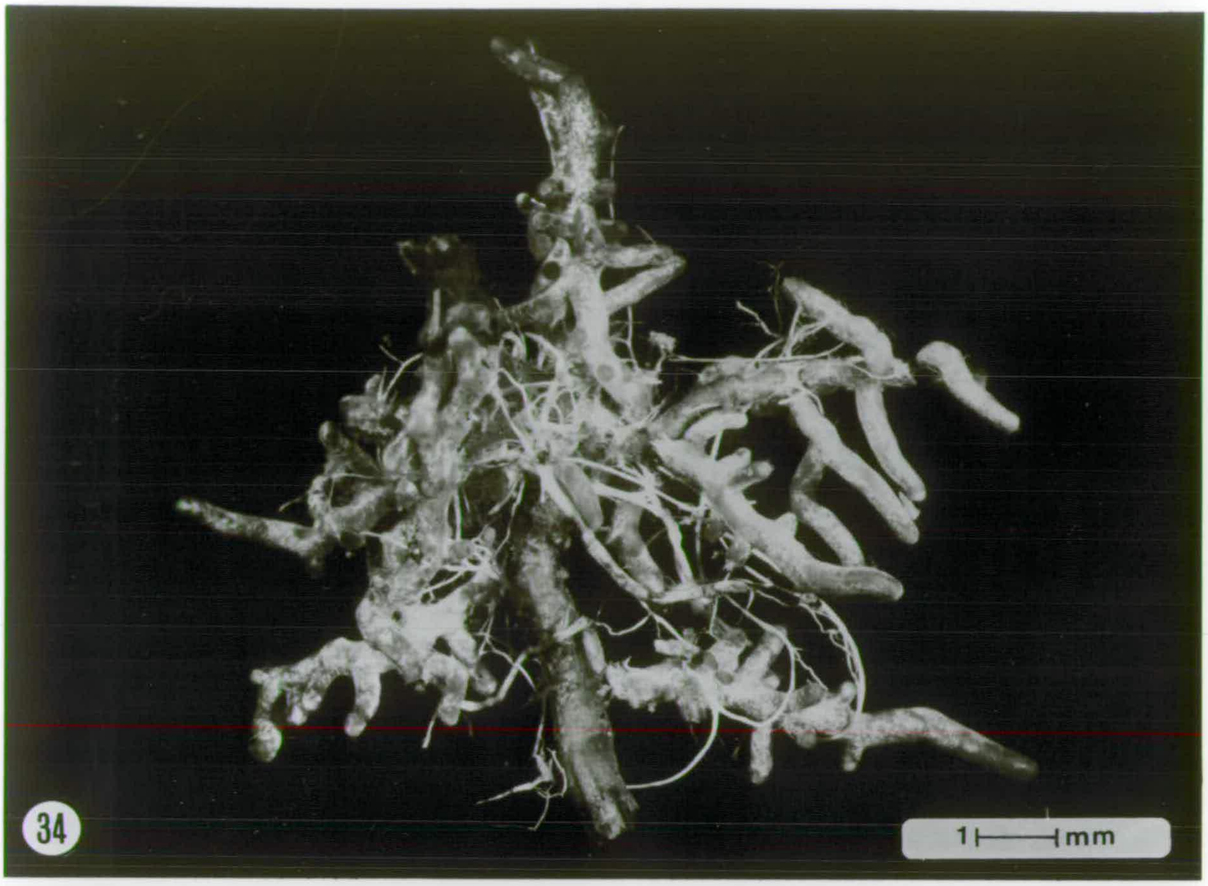

33

Leccinum spp. Plates 6, 34, 35, 36.

Basis of identification	Pure culture synthesis; association with fruitbodies.
Gross morphology	Elements tortuous, thick (0.3 - 0.5 mm wide) with rounded tips and frequent branches; with age dense clusters of mycorrhizas may form (Plate 34).
Sheath	Silvery white in colour when young (Plate 6) turning fawn, with silvery patches, with age; 12-13 µm thick, with reticulate surface mycelium (Plate 35).
Extramatrical mycelium	Hyphae 2.5 - 3 µm wide, extending up to 80 µm from the sheath, most commonly on older mycorrhizas; no clamp connections observed.
Mycelial strands/ sclerotia	Prominent white mycelial strands (Plates 6, 34), sometimes yellowing with age; generally 30-120 µm diameter but strands near the base of a fruitbody can be 0.8 mm diameter; strands smooth and regular in appearance and surface hyphae may be verrucose (plate 36; *L. roseofracta* Watling). No sclerotia observed.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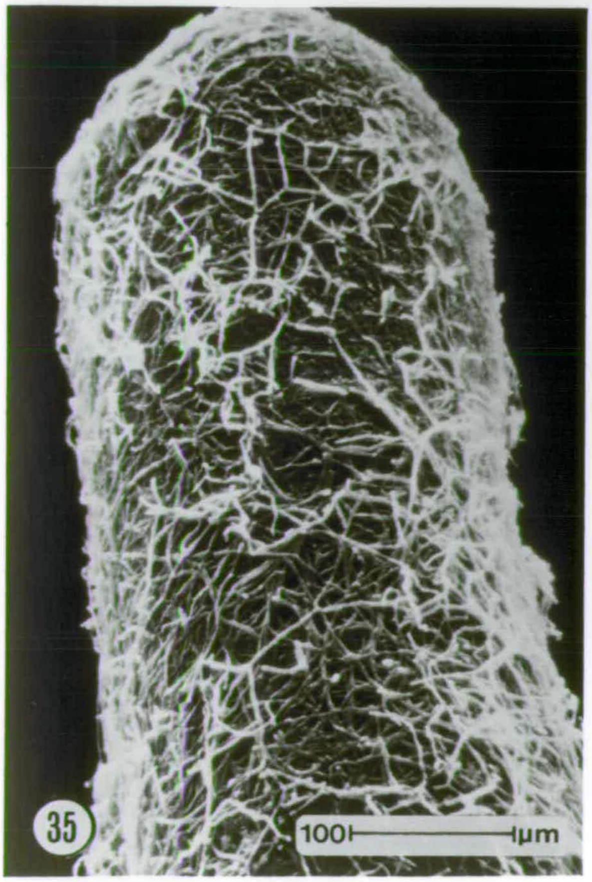
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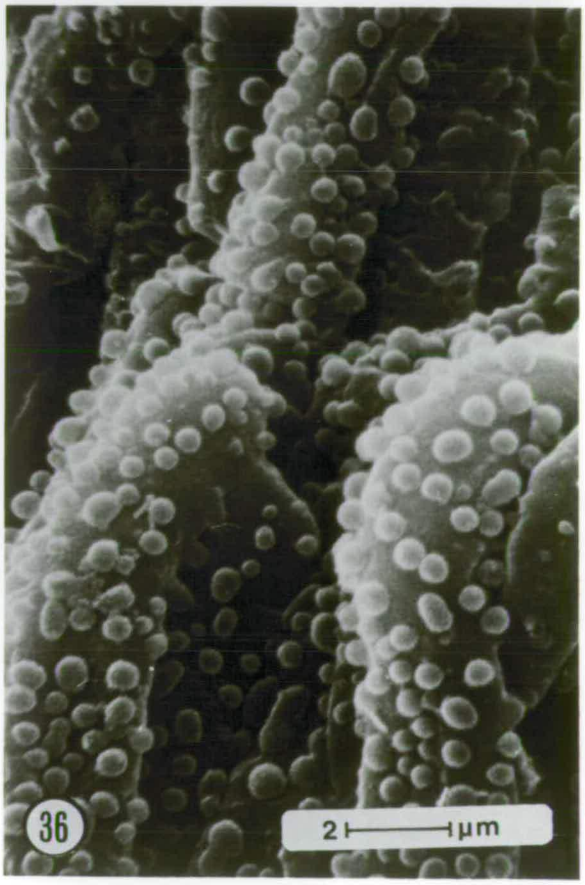

36

Thelephora terrestris (Ehrh.) Fr. Plates 7, 37, 38, 39.

Basis of identification	Pure culture synthesis; association with fruitbodies; seedling assay of inoculum-supplemented soils.
Gross morphology	Long (up to 7 mm), thin (up to 0.3 mm wide), slightly tortuous, generally unbranched or sparsely branched (Plate 37).
Sheath	Milky white initially with occasional silvery patches, darkening with age to pink/brown or even deep chocolate brown (Plate 7); all three colours may be present, in patches, on individual elements. Sheath thin (9-10 µm) with a smooth surface.
Extramatrical mycelium	Well-spaced, thin (1.5 - 3 µm), projecting hyphae may extend up to 160 µm from the sheath surface (Plate 39); clamps frequent and usually present on the basal septum of each hypha.
Mycelial strands/ sclerotia	No sclerotia observed. Mycelial strands present, but more frequent in pure culture syntheses than on mycorrhizas in soil unless fruitbodies are present; strands up to 56 µm wide, coloured like the sheath, loosely organised, with a fringe of surrounding hyphae (Plate 38).


37

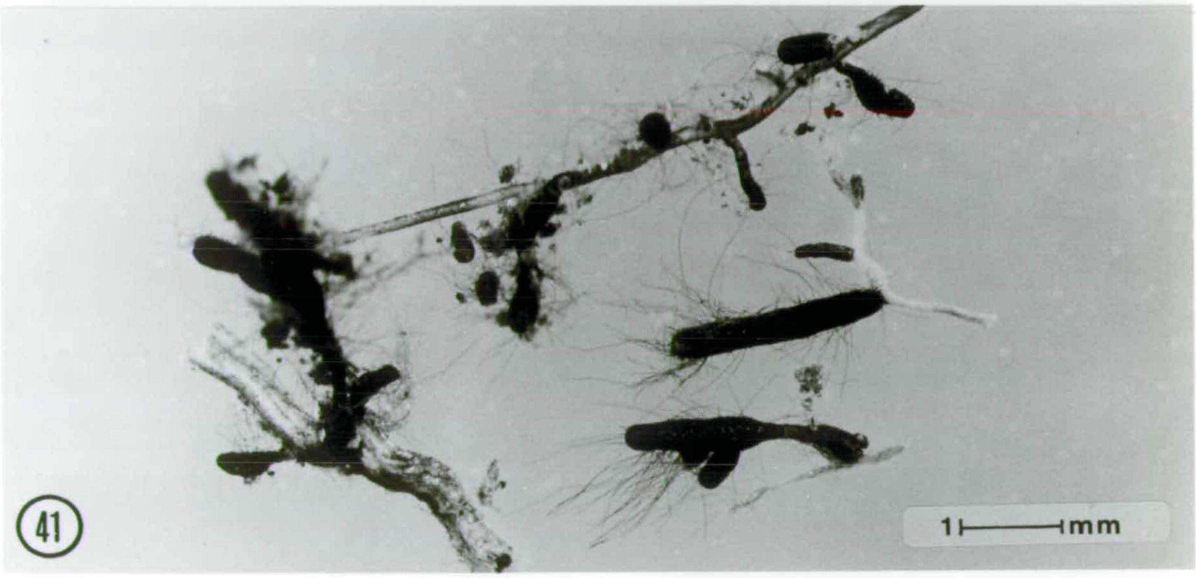

38

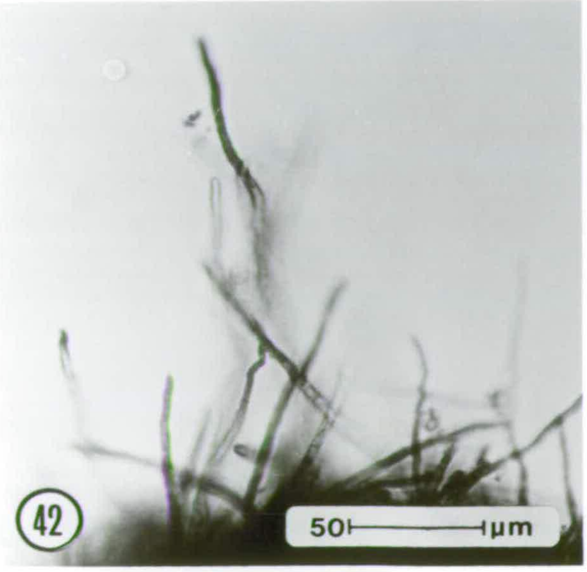

39

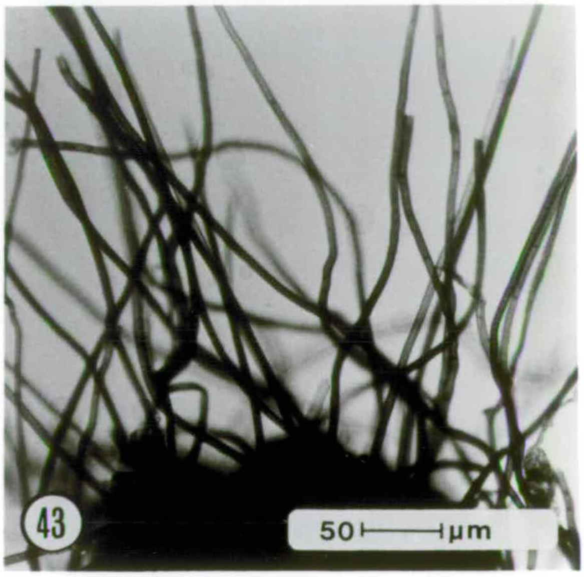
Elaphomyces granulatus Fr.	Plates 8, 40, 42.
Basis of identification	Association with fruitbodies.
Gross morphology	Individual elements irregularly swollen (up to 0.4 mm wide) and tortuous (Plate 8) with frequent branches; mycorrhizas form dense tightly packed clusters which may completely surround the ascocarps (Plate 40).
Sheath	Smooth, pale orange/yellow (Plate 8); yellow 'flecks' of material observed in mycorrhizal clusters (Plate 40).
Extramatrical mycelium	Patchy hyphae, 3 µm wide and up to 120 µm long (Plate 42).
Mycelial strands/ sclerotia	None observed.

Cenococcum geophilum Fr.	Plates 41, 43
Basis of identification	Published descriptions (e.g. Pigott, 1982a).
Gross morphology	Mycorrhizas simple, 0.3 mm wide and up to 2.5 mm long, generally borne singly but may bear one or two branches (Plate 41).
Sheath	Black, shiny, with smooth sheath surface.
Extramatrical mycelium	Thick, black, projecting bristle-like hyphae surround the mycorrhiza (Plate 43); they are 4.6 µm wide and up to 1.8 mm long.
Mycelial strands/ sclerotia	No mycelial strands observed. Spherical, hard, smooth black sclerotia occasionally observed, up to 2 mm diameter.

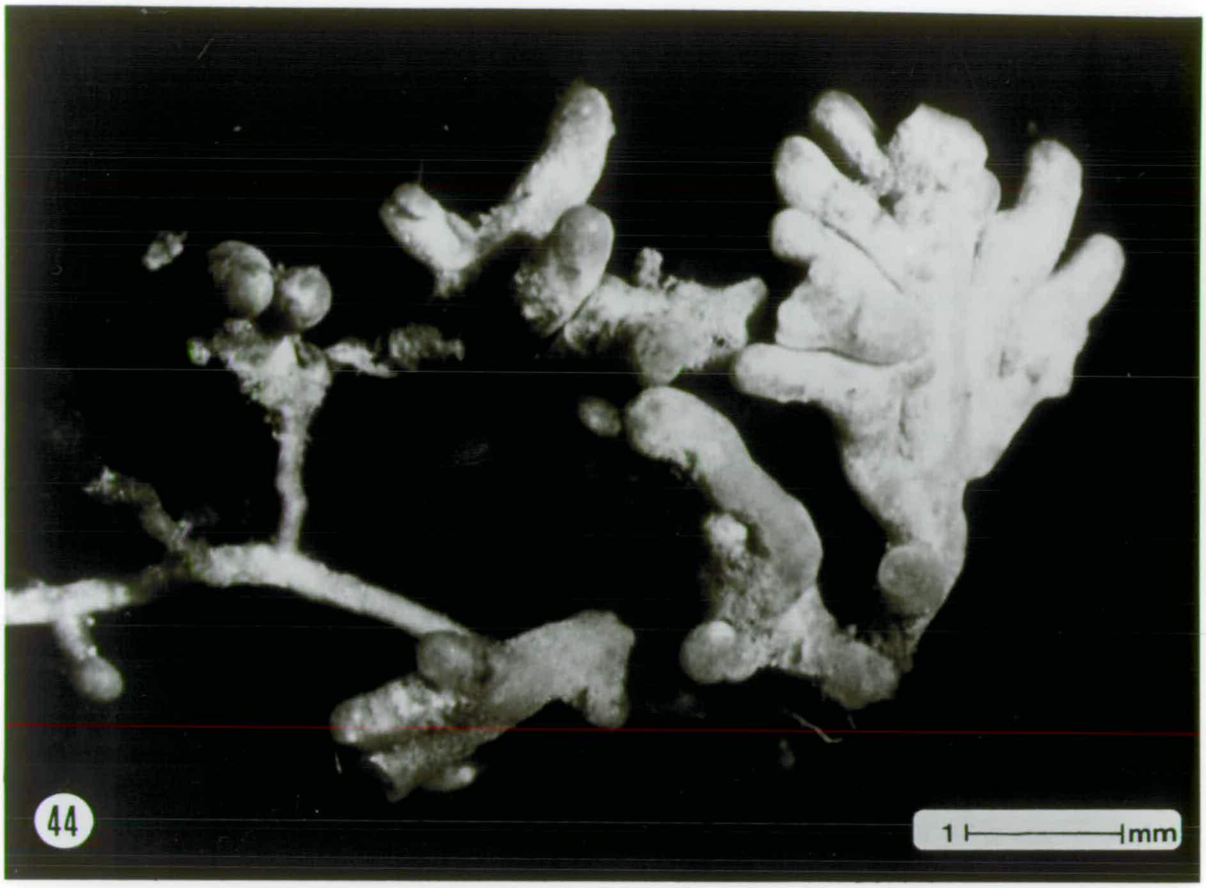

40
1 mm


41
1 mm


42
50 µm


43
50 µm

Type 1	Plates 44, 45, 46.
Gross morphology	Thick, swollen mycorrhiza with blunt rounded tips (Plate 44); individual elements up to 0.4 mm diameter, but where branches coalesce a single mycorrhizal element may measure up to 1 mm in width.
Sheath	Thick (25-30 µm), surface hyphae reticulate, white with silvery 'hoarfrost' appearance.
Extramatrical mycelium	Sparse, where present the hyphae are short (30 µm long, 3 µm wide) with short bulbous branches (Plate 45).
Mycelial strands/ sclerotia	Mycelial strands frequent, smooth, yellow branched, up to 40 µm diameter (Plate 46). No sclerotia observed.



44

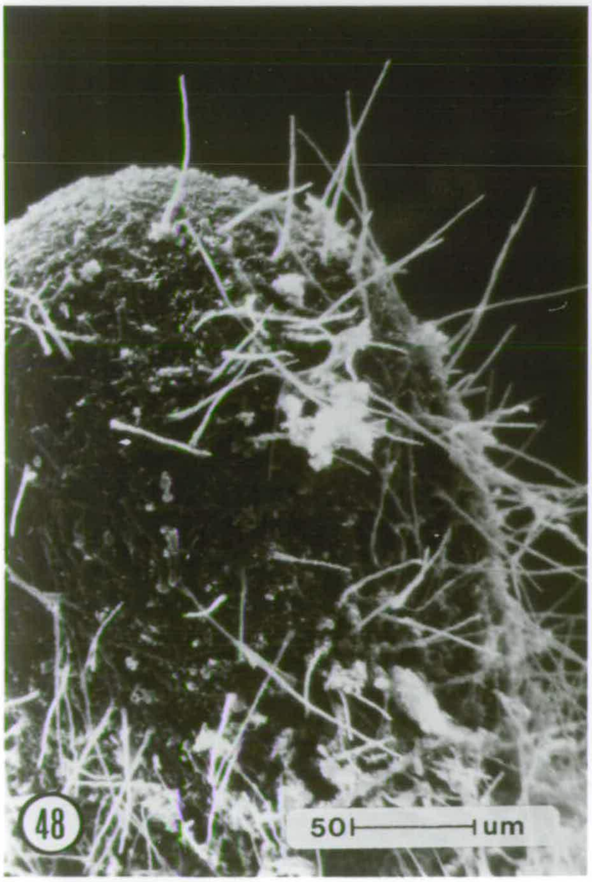
45

46

Type 2. 'Brown with cream tips' Plates 9, 47, 48, 49.

Gross morphology	Thick, swollen mycorrhiza with rounded tips; shorter elements tend to have an even diameter (0.24 - 0.4 mm) but longer elements (up to 5 mm) are more irregular and tortuous. Branches frequent, usually short, rarely exceeding $\frac{1}{3}$ of the length of the parent (Plate 47).
Sheath	Thick (22-31 µm), surface smooth with a distinct jigsaw appearance when viewed by light microscopy; chestnut-brown with paler tips even on old mycorrhizas; on young actively growing mycorrhizas the tips are creamy-yellow (Plate 9).
Extramatrical mycelium	Evenly spaced, straight unbranched 'stiff' projecting hyphae (Plates 48, 49), up to 100 µm long and 1.5 µm wide. These hyphae may be absent from the tips (Plate 48) and older regions of mycorrhizas. No clamps observed.
Mycelial strands/ sclerotia	None observed.

47



48

49

Type 3. Plates 50, 51, 52.

Gross morphology	Individual elements sinuous, sometimes very long (up to 1 cm), generally of even diameter (0.3 - 0.4 mm); branches frequent but irregularly spaced (Plate 50).
Sheath	White to pale fawn, 12.5 - 16.5 μm thick, surface of loosely organised hyphae but invariably obscured by the surrounding mycelium.
Extramatrical mycelium	Dense mass of hyphae concolorous with the sheath, and in places loosely organised into mycelial wefts (Plate 51) which are usually white, up to 0.1 mm diameter but sometimes appearing up to 0.8 mm diameter because of intermingling with unorganised hyphae, these are branched, 2 - 2.5 μm wide, with granular cytoplasm and clamp connections (Plate 52).
Mycelial strands/ sclerotia	None observed.

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(51)

(52)

Type 4. Plate 53.

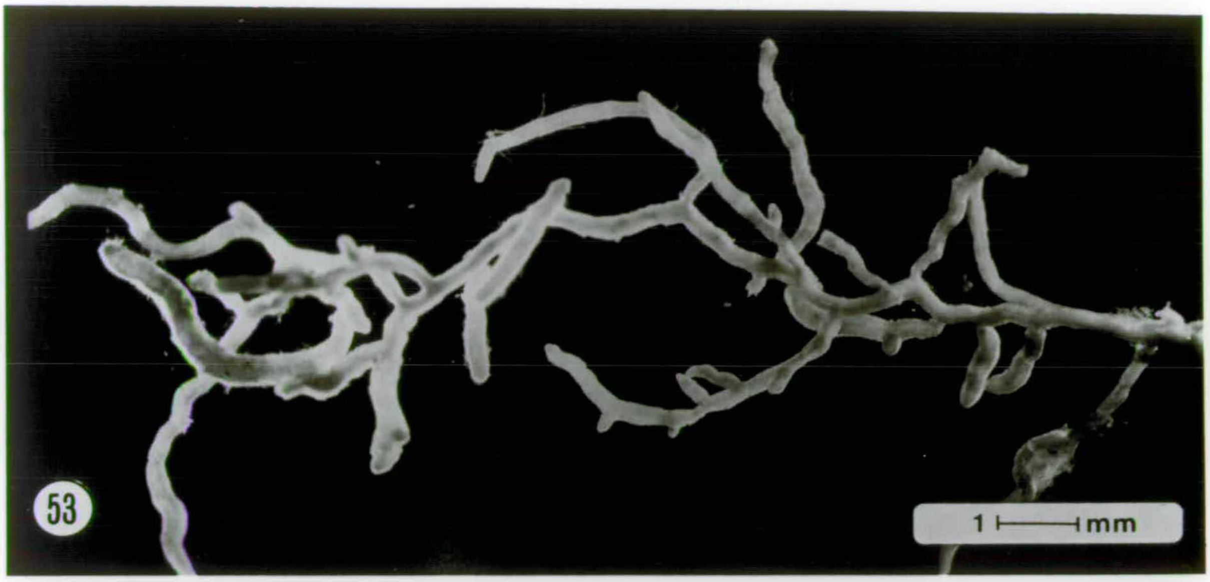
Gross morphology	Long, thin mycorrhiza, individual elements are tortuous and frequently beaded, 0.2-0.3 mm wide and 6-7 mm long; branches frequent but irregularly spaced.
Sheath	Off-white turning pale fawn with age, showing surface network of hyphae but no projecting mycelium has been observed.
Extramatrical mycelium	None observed.
Mycelial strands/ sclerotia	None observed.

Type 5. Plate 54

Gross morphology	Short, stubby mycorrhiza; individual elements straight to slightly tortuous, 0.35 mm wide, up to 4-5 mm long, with blunt rounded tips; branches frequent.
Sheath	White when fresh turning pale grey to brown, with velvety surface.
Extramatrical mycelium	Projecting hyphae uniformly covering the mycorrhiza; hyphae 3-12 µm wide and 120-130 µm long, generally unbranched with frequent clamp connections.
Mycelial strands/ sclerotia	None observed.

Type 6. Plate 55

Mycorrhizas generally borne singly or with an occasional short branch. This type resembles Hebeloma-type mycorrhizas (see earlier) in most respects but it is distinguished because of the prominent golden-yellow colour of the sheath surface and hyphae.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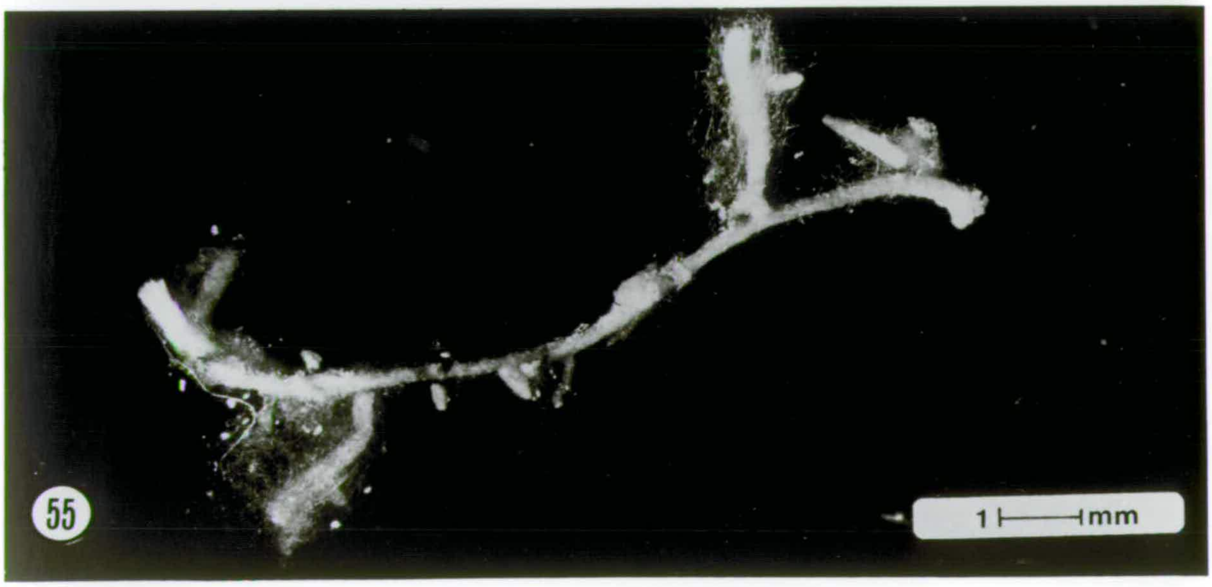
54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Type 7. Plate 56.

Gross morphology	Short, stumpy mycorrhizas, generally borne singly or with occasional single branches; individual elements 2-4 mm long and up to 0.4 mm wide.
Sheath	Pink-brown with silvery sheen and reticulate surface hyphae.
Extramatrical mycelium	Sparse, where present hyphae are 3 μm wide and up to 40 μm long, with infrequent clamps.
Mycelial strands/ sclerotia	None observed.

Type 8. Plate 57

Gross morphology	Individual elements straight to slightly tortuous and of uniform diameter (0.4 mm), up to 6 mm long. Frequent branches forming tight clusters.
Sheath	Initially silver turning a distinct lilac-purple with reticulate surface mycelium.
Extramatrical mycelium	Extending out to 1 mm from the sheath; composed of frequently branched hyphae, 3 μm wide with occasional clamps.
Mycelial strands/ sclerotia	None observed.

Type 9. Plate 58

Gross morphology	Individual elements slightly tortuous, up to 0.4 mm wide and 7 mm long; mycorrhizas may branch frequently or remain as long slender single elements.
Sheath	Initially silvery but becoming pink due to red encrustations on the outermost hyphae of the reticulate sheath surface.
Extramatrical mycelium	Hyphae, 2.5 - 3 μm wide and up to 150 μm long.
Mycelial strands/ sclerotia	No sclerotia observed. Mycelial strands present, silver and 15-20 μm wide.

56

57

58

Type 10. Plate 59.

Gross morphology	Mycorrhizas usually single but occasionally branched; individual elements generally 0.4 mm wide but can be up to 0.6 mm wide.
Sheath	White/grey with silver 'hoarfrost' appearance, reticulate surface mycelium.
Extramatrical mycelium	Occasional patches of narrow (2 µm wide) hyphae extending to 120 µm from the sheath; the hyphae bear repeating arbuscular branching systems near their tips.
Mycelial strands/ sclerotia	None observed.

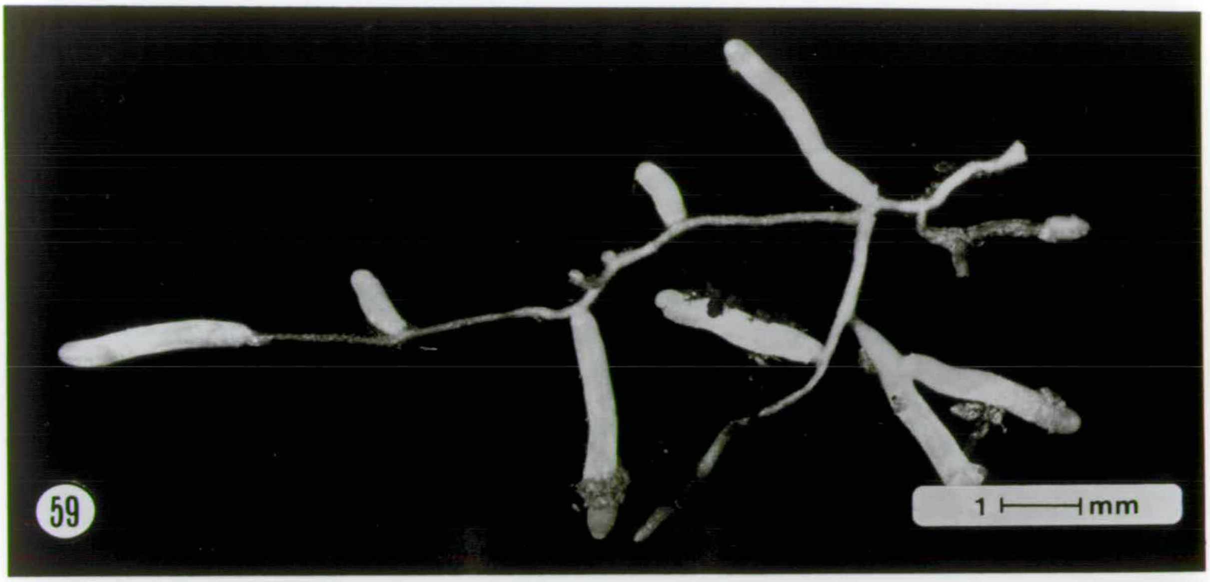
Type 11. Plate 60.

Gross morphology	Individual elements slightly tortuous, generally of uniform diameter (0.25 - 0.35 mm) and up to 5 mm long; branches may give a regular pinnate appearance.
Sheath	Smooth, reddish-brown turning darker with age.
Extramatrical mycelium	Covering of short (<30 µm long) hyphae of variable diameter (1.6 µm), no clamps observed.
Mycelial strands/ sclerotia	None observed.

Type 12. Plate 61.

Gross morphology	Branches develop with age giving a regular pinnate or doubly-pinnate appearance. Individual elements generally straight and of uniform diameter (0.4 mm).
Sheath	Smooth, orange-brown.
Extramatrical mycelium	Occasional single hyphae, 25 µm long and 3 µm wide.
Mycelial strands / sclerotia	None observed.
Notes	This mycorrhizal type is similar to some Lactarius-type mycorrhizas.

59



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61

Type 13.	Plate 62.
Gross morphology	Predominantly borne singly but older mycorrhizas may have up to four evenly spaced branches; individual elements club-shaped when young, 0.3 mm diameter and up to 1 mm long, older mycorrhizas are more tortuous.
Sheath	Smooth, reddish-brown.
Extramatrical mycelium	Coat of radiating, unbranched, silky, yellow hyphae that extend up to 0.8 mm from the mycorrhiza and are up to 4.8 µm in diameter; no clamps observed.
Mycelial strands/ sclerotia	None observed.

Type 14.	Plate 63.

This type is similar to type 5 mycorrhizas except that the extramatrical hyphae are narrower, up to 2 µm diameter, and clamps are observed only very infrequently.

Type 15.	Plate 64.
Gross morphology	Club-like, generally under 3 mm long, up to 0.4 mm wide, with occasional short branches.
Sheath	Off-white to pale fawn; young, fresh mycorrhizas may have a silvery sheen.
Extramatrical mycelium	Short dense covering of narrow hyphae (1.5 µm diameter) up to 100 µm long.
Mycelial strands/ sclerotia	None observed.
Note	Numerous soil particles adhere to the hyphae giving the mycorrhiza a 'dirty' appearance, these soil particles have to be teased off to observe the sheath.

62

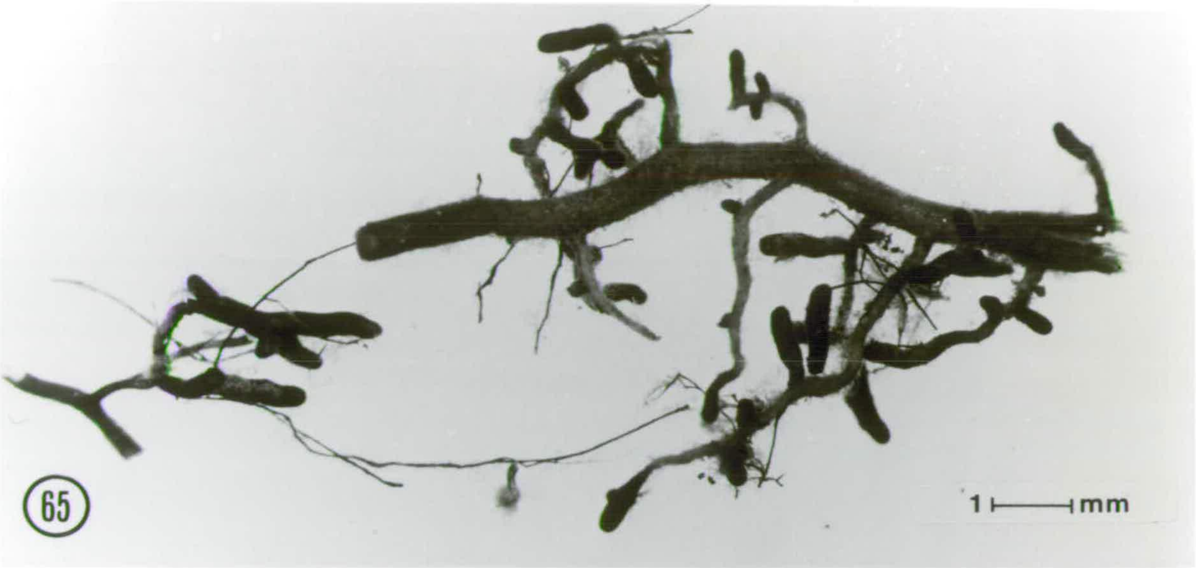
63

64

Type 16.	Plate 65.
Gross morphology	Mycorrhizas generally borne singly but may have occasional short branches; single mycorrhizas club-shaped, 0.2 mm diameter and usually <2.5 mm long, older mycorrhizas being longer and more tortuous.
Sheath	Black, granular appearance.
Extramatrical mycelium	Occasional narrow (1.5 µm) hyphae project up to 40 µm from the sheath.
Mycelial strands/ sclerotia	No sclerotia observed. Mycelial strands frequent, black, surrounded by a fringe of hyphae some of which bear clamp connections.

Type 17.	Plate 66.
Gross morphology	Individual elements straight to slightly tortuous, up to 0.4 mm diameter and 4 mm long; frequent regular pinnate branching.
Sheath	Black, with granular appearance.
Extramatrical mycelium	Mycorrhiza covered with radiating, unbranched, reddish silky hyphae (4 µm diameter) up to 0.5 mm long.
Mycelial strands/ sclerotia	No sclerotia observed. Thick, black mycelial strands, up to 60 µm wide and covered with similar hyphae to those around the sheath.

Type 18.	Plate 67.
Gross morphology	Usually borne singly but often having a single branch at the base giving a bifurcate appearance; 2.5 mm long and 0.4 mm wide.
Sheath	Fawn, 'velvety' due to surrounding mycelium.
Extramatrical mycelium	Irregular cover of branched hyphae, 3-5 µm wide and 60 µm long, clamps present.
Mycelial strands/ sclerotia	No sclerotia observed. Strands yellow branched, loosely organised, up to 30 µm in diameter.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Succession of mycorrhizal fungi on birch: infection of seedlings planted around mature trees

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Summary Non-mycorrhizal seedlings of *Betula pendula* were planted around an 11-year old tree of *B. pubescens* in an experimental plot at Bush Estate, south of Edinburgh. Half (23) of the seedlings were in untreated planting positions and half (24) in positions that had been cored to sever connections of the roots with the parent tree. After 17 weeks, seedlings in the non-cored positions bore mycorrhizas mainly of *Lactarius pubescens* whereas mycorrhizas of this species were uncommon on seedlings in cored positions. Much smaller and usually non-significant differences were found with respect to other mycorrhizal types in cored and non-cored positions. It is concluded that *L. pubescens* infected seedling roots by means of mycelial strands which must remain attached to the parent tree (food base) in order to infect. The significance of the results for the concept of early-stage and late-stage mycorrhizal fungi is discussed.

Introduction

A succession of mycorrhizal fungi has been reported on roots of birch (*Betula pendula* Roth. and *B. pubescens* Ehrl.)[2,6,7]. In this respect a distinction has been made between early-stage and late-stage fungi, because the former can infect seedlings grown in cores of soil taken beneath their fruitbodies whereas the latter cannot do so[2,6]. A common feature of the reported late-stage fungi, like *Lactarius pubescens* Fr. and *Leccinum* spp., is that they form conspicuous mycelial strands. Most work on such mycelial strands has concentrated on their abilities to absorb and translocate materials from the external environment to the plant root (*e.g.* reference 3). But Garrett[4] considered that the major role of mycelial strands and rhizomorphs in root-infecting fungi as a whole was as agents of infection. The possibility that strands serve this role in mycorrhizal fungi has been recognized by several workers (*e.g.* reference 1) but never conclusively demonstrated.

Because the infectivity of mycelial strands and rhizomorphs depends on nutrients supplied by a food base[5] it was thought possible that the failure of late-stage mycorrhizal fungi to infect seedlings in soil cores was a result of the severence of inoculum in these cores from the food base provided by the parent tree. An experimen. was done to investigate this.

Experimental

An 11-year old tree of *B. pubescens* was selected in an experimental plot at Bush Estate, south of Edinburgh, where the distributions of mycorrhizas and fruitbodies had been recorded in previous years[7]. The tree had mycorrhizas of *L. pubescens* more or less evenly distributed around it to a distance of at least 1 metre from the trunk. The ground beneath the tree was covered with black polyethylene sheeting from December 1980 until May 1981, to suppress growth by herbaceous plants. Meanwhile, non-mycorrhizal seedlings of *B. pendula* were grown in vermiculite-peat in laboratory conditions[8], hardened-off in a cold-frame and prepared for transplanting into the field when they were 55 days old. They were then 20.1 ± 1.75 (S.E.) mm tall, weighed 3.6 ± 0.51 mg (oven-dry) and bore 49.3 ± 5.26 uninfected root tips.

Forty-eight planting positions were chosen, in four annuli around the tree, at 20, 35, 50 and 75 cm radii from the trunk. The planting positions were evenly spaced within the annuli, so there were progressively more seedlings further out from the tree. Alternate positions were untreated and received one seedling each – 'non-isolated'. At other positions a core of soil 8 cm deep and of 10 cm diameter was taken with a corer and then replaced with minimal disturbance, knowing that roots within the core had been severed from the parent tree. Seedlings were then planted – 'isolated'. All plantings were done on 3 June 1981 by removing a narrow core of soil with a cork-borer (1 cm diameter) and inserting into the hole a root system of a seedling grown in a 1 cm diameter plastic sleeve (the sleeve being removed at planting)[8].

The seedlings were watered as required in the summer of 1981, and a 2 cm deep mulch of sphagnum peat was applied to the site to help conserve moisture. At intervals the cores containing the 'isolated' seedlings were carefully lifted and replaced immediately, to prevent re-invasion by roots or mycelial strands. Light intensities at seedling level, and temperatures and rainfall were recorded, and the positions of fungal fruitbodies were recorded as they appeared.

Seedlings were sampled 17 weeks after they had been planted into the field, when the isolated seedlings were both taller (39.1 ± 1.42 mm) and heavier (37.6 ± 3.93 mg dry wt.) than the non-isolated ones (34.1 ± 1.43 mm and 27.5 ± 3.53 mg). The root systems were washed and assessed for mycorrhizal types, distinguished on the basis of colour, gross morphology and microscopical features, using as standards mycorrhizas formed in bixenic culture or consistently associated with fruitbodies in the field (Table 1).

Results

There were no significant differences in (a) total numbers of root tips and (b) numbers of uninfected root tips between isolated and non isolated seedlings (Table 1). Four predominant mycorrhizal types were seen on the seedling root systems. Unidentified types '4' and '5' were found on similar numbers of isolated and non-isolated seedlings, and the numbers on these seedlings did not differ significantly (Table 1). By contrast, Lactarius-type mycorrhizas were present on significantly more non-isolated than isolated seedlings (Table 1) and they were frequently the dominant type on non-isolated seedlings (in 11 out of 23 instances) but were never dominant on isolated seedlings. The difference in the case of Lactarius-type mycorrhizas can be illustrated in another way: a total of 73 mycorrhizas of this type was recorded on the 24 isolated seedlings, compared with 1469 on the 23 non-isolated seedlings (one of the original 24 seedlings in this category died).

Hebeloma-type mycorrhizas were present on many non-isolated seedlings but

Table 1. Occurrence of mycorrhizal types on birch seedlings grown for 17 weeks in isolated (cored) * and non-isolated positions around a mature birch tree

Mycorrhizal type	No. seedlings bearing each mycorrhizal type (no. with > 20% root tips of that type in parentheses)		Mean no. root tips of each mycorrhizal type (means of log × + 1 in parentheses)		
	Isolated (Max. 24)	Non-isolated (Max. 23)	Isolated	Non-isolated	LSD (P = 0.05)
Hebeloma	6 (3) **	19 (4)	16 (0.35)	18 (0.89)	(0.39)
Lactarius	3 (2) ***	22 (17)	3 (0.15)	64 (1.66)	(0.32)
Type 4	17 (16)	11 (11)	70 (1.33)	34 (0.87)	(0.50)
Type 5	19 (13)	17 (9)	54 (1.28)	47 (1.12)	(0.49)
Total no. mycorrhizal root tips/seedling			143	166	
Total no. uninfected root tips/seedling			11	7	
Total no. root tips/seedling			154	173	

* For explanation see text

** Difference significant at $P = 0.01$

*** Difference significant at $P = 0.001$

not on the isolated seedlings. In most cases, however, they were present in very low numbers and if the value of 20% of total root tips represented by Hebeloma-type mycorrhizas is taken as an indication of good establishment on a seedling (even this is much less than would be true of a dominant mycorrhizal type) then Table 1 shows that few seedlings in either category were heavily colonized by it. In fact, there was no difference between treatments in the number of Hebeloma-type mycorrhizas: 389 were recorded on the 24 isolated seedlings, compared with 408 on the 23 non-isolated seedlings.

The distribution of mycorrhizal types on the parent tree root system was examined early in November 1981 by taking small cores of soil (8 × 2 cm diameter) at 25 cm distance intervals on ten radiating transects from the tree trunk. Lactarius-type mycorrhizas represented only 12% of the total number of mycorrhizal root tips, the highest recorded percentage being 31.0 for the ten cores at 100 cm from the trunk. Type '5' was the dominant type, representing 48.8% of root tips overall. Hebeloma-type represented 26.1% type '4' 9.9% and other types 3.2%. Thus, comparing the results for seedlings and the parent tree root system, Lactarius was markedly over-represented on the non-isolated seedlings and under-represented on the isolated seedlings, Hebeloma and type '5' were under-represented on the seedling roots in general, and type '4' was over-represented on the seedling roots in general.

Most fruitbodies that appeared around the parent tree in autumn 1981 were of *L. pubescens*, *Hebeloma velutipes* Bruchet and *Cortinarius* sp. Three species of *Russula* were also seen (*R. versicolor* J. Sch., *R. betularum* Hora and *R. grisea* (Secr.) Gill) but neither they nor the *Cortinarius* sp. seemed to represent the unidentified mycorrhizal types on the roots.

Discussion

L. pubescens was the only one of the mycorrhizal types observed that formed mycelial strands; indeed, it was the only late-stage type (in the sense defined in reference 2) that occurred on the seedlings, *Russula* and *Cortinarius* spp. being excluded from consideration because they apparently did not infect seedling roots.

The results show clearly that Lactarius can establish mycorrhizas on seedlings in soil, provided that the fungus is not separated from the parent tree; furthermore, it can colonize the seedling roots to a greater degree than its level of establishment on the parent tree root system would suggest. This is consistent with the view that Lactarius colonized the seedling roots by means of mycelial strands or, at least, by means of hyphae that need to remain attached to the food base provided by the parent tree. In this respect the results parallel those previously obtained for root-infecting pathogens, like *Armillaria mellea* (Vahl ex Fr.) Kummer[5].

Supporting evidence for the view that mycelial strands are important in colonization of seedling roots by late-stage mycorrhizal fungi has recently been obtained. Naturally occuring birch seedlings were collected in a chestnut (*Castanea sativa* Mill.) coppice with oak standards near Rochester, Kent, late in July 1981. The seedlings were less than one year old but 73% of them bore a late-stage mycorrhizal type associated with thick yellow mycelial strands and resembling some mycorrhizas formed by the Boletaceae. Soil in which these seedlings were growing was taken to a glasshouse and sown to birch, but only the usual range of early-stage mycorrhizal types occurred on the seedling roots. This could be interpreted as evidence that seedlings developing around parent trees or their living coppice stools in the field are infected by late-stage mycorrhizal fungi but that isolation of these fungi from the parent tree leads to failure to infect seedlings.

The results of this study raise interesting questions about the respective roles of early- and late-stage mycorrhizal fungi. It is unclear at present if early-stage mycorrhizal fungi have a significant role in the establishment of naturally regenerating tree seedlings in the woodland environment.

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