

Diversity of ectomycorrhizas in old-growth and mature stands  
of Douglas-fir (*Pseudotsuga menziesii*) on southeastern  
Vancouver Island

by

Douglas Mark Goodman  
B.Sc., University of Victoria, 1980  
B.Sc., University of Victoria, 1985  
M.Sc., University of Guelph, 1988

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We accept this dissertation as conforming  
to the required standard

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Dr. ~~J.N.~~ Owens, Supervisor (Department of Biology)

---

Dr. ~~J.A.~~ Trófymow, Co-Supervisor, Additional Member  
(Department of Biology)

---

Dr. ~~B.J.~~ Hawkins, Departmental Member (Department of  
Biology)

---

Dr. ~~J.~~ Antos, Departmental Member (Department of Biology)

---

Dr. ~~(M.C.R.)~~ Edgell, Outside Member (Department of Geography)

---

Dr. ~~(S.)~~ Bérch, Additional Member (Research Branch, British  
Columbia Ministry of Forests)

---

Dr. ~~(R.M.)~~ Danielson, External Examiner (Department of  
Biological Sciences, University of Calgary)

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University of Victoria

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Supervisor: Dr. John N. Owens

ABSTRACT

Concern about potential losses of biological diversity and productivity following clear-cut logging of old-growth forests in British Columbia led me to compare ectomycorrhizas in old-growth and mature stands of Douglas-fir.

Two sites were selected, each with an old-growth (288-, 441-yr-old) and a mature stand (87-, 89-yr-old) well-matched in tree species, soil and topography. A total of 120 soil cores  $\leq 15$  cm deep by 5 cm diameter were taken at random from four 60 m square plots (one per stand). Samples were taken in spring and fall at each site. All morphological types of ectomycorrhizas in one half of each core were counted and characterized in detail. Ectomycorrhizal abundance and frequency was compared in logs, stumps, the forest floor over bedrock or gravel, the forest floor near the base of trees, the forest floor elsewhere, and mineral soil.

Old-growth and mature stands were very similar in richness, diversity and types of ectomycorrhizas. Sixty-nine types of ectomycorrhizas were described. Nineteen types each accounted for more than one percent of the 17,500 ectomycorrhizal root tips examined, and eighteen types were found in five or more of the 120 soil cores. Extrapolation indicates a total richness of roughly 100 types in the four plots. Co-dominant fungi were *Cenococcum geophilum* Fr. (24% of all ectomycorrhizal root tips), a *Rhizopogon* Fr. of the section *villosuli* (10%), *Hysterangium* Vitt. (9%), *Lactarius*

*deliciosus* (Fr.) S.F.G. (6%), and *Piloderma fallax* (Libert) Stalpers (4%). *Cenococcum geophilum*, *Rhizopogon* Fr. and *L. deliciosus* were abundant in both mineral soil and organic substrates, *Piloderma fallax* was associated with decayed wood, and *Hysterangium* and type 27 were in organic substrates only. A bright greenish-yellow felty type was found in 5 cores in mineral soil only.

The similarity of the ectomycorrhizal communities of old-growth and mature stands was probably due to their proximity (< 200 m apart) and the similarity of their vegetation and soil. Differences may occur at some sites if ectomycorrhizal succession has been delayed or redirected as a result of frequent or severe disturbance, lack of old-growth legacies (logs and stumps), or lack of old-growth stands from which fungi can disperse.

EXAMINERS

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Dr. J.N. Owens, Supervisor (Department of Biology)

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Dr. J.A. Trofymow, Co-Supervisor, Additional Member  
(Department of Biology)

---

Dr. B.J. Hawkins, Departmental Member (Department of  
Biology)

---

Dr. J. Antos, Departmental Member (Department of Biology)

---

Dr. M.C.R. Edgell, Outside Member (Department of Geography)

---

Dr. S. Berch, Additional Member (Research Branch, British  
Columbia Ministry of Forests)

---

Dr. R.M. Danielson, External Examiner (Department of  
Biological Sciences, University of Calgary)

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## General introduction

**1. Biological diversity in forest soils.** Many British Columbians have concerns about loss of biological diversity and productivity following clear-cut logging. Kellert (1986) described the value of biodiversity as recreational, ecological, moral, scientific, aesthetic, utilitarian and cultural. Ecologists have been interested in the relationship between biodiversity and the stability, and functioning of ecosystems. The importance of biodiversity for the stability of an ecosystem is questionable (Dempster and Coaker 1974). Yet diversity enhances the stability of some communities (Way 1977), and ecosystems will become unsustainable if modified too much in certain ways (Holdgate 1991). Examples from agriculture show that increased diversity of bacteria or fungi in soil can prevent crop losses caused by destruction of roots by pathogenic fungi (Alabouvette 1986, Campbell 1989, Malaczuk 1979).

Forest soils are generally less fertile than agricultural soils, and rely on nutrient mobilization in a surface organic layer that is characterized by a greater diversity of micro-organisms than in agricultural soils (Pritchett and Fisher 1987). Long-term productivity (ie., sustainability) of forests will require maintenance of soil function, which in turn involves a wide range of soil organisms. Yet we do not understand the impact of losses of biodiversity in disturbed forest soils. Forest managers need to know the long-term effects on soil characteristics of clear-cutting, site preparation, brushing, reforestation options, thinning, fertilization and other practices. Hence the value of comparing mycorrhizas in natural old-growth stands with mycorrhizas in second-growth stands with a documented management history.

**2. Practical importance of understanding the ecology of ectomycorrhizas.** Ectomycorrhizas are essential interfaces between soil and roots of Douglas-fir. Nutrients and water that are responsible for the growth of Douglas-fir trees pass through ectomycorrhizal fungi before they enter the

vascular system of roots and are translocated. Researchers have recently been investigating the roles of individual species of ectomycorrhizal fungi in forest soils (e.g. Abuzinadah and Read 1986a, 1986b, 1989a, 1989b, Cromack 1990, Finlay and Frostegard 1990). Further study is needed to determine the soil conditions favourable for individual ectomycorrhizal fungi, the substrates they exploit, and whether some ectomycorrhizal fungi are more important to forest health than others.

Perry (1985), and Schoenberger and Perry (1982) have suggested that diversity of ectomycorrhizas is important for the stability of Douglas-fir ecosystems. As a stand of trees ages, there are changes in the pathways of nutrient cycles in the soil, often accompanied by a succession of ectomycorrhizal fungi. Old-growth stands may be essential for the survival and dispersal of those mycorrhizal fungi adapted to soil conditions of older stands. If older stands are important as sources of inoculum for the dispersal of ectomycorrhizal fungi into younger stands, this could have an implication for the size and arrangement of cut-blocks. Knowing habitat requirements of ectomycorrhizal fungi could influence choices of practices that affect fertility of soil, structure of soil and amounts and sizes of coarse, woody debris.

The central question to be answered by this project was whether old-growth and second-growth stands of Douglas-fir differ significantly in abundance, diversity and species composition of ectomycorrhizas. Further, if differences are observed, are they related to or do they result from differences in soil characteristics? Comparison of diversity of ectomycorrhizas of Douglas-fir in old-growth and mature stands will help us assess the impact on biodiversity of harvesting old growth in the Coastal Douglas-fir biogeoclimatic zone and will indicate whether ectomycorrhizal succession occurs in stands over 80-yr-old.

Relating ectomycorrhizal distribution to soil characteristics may suggest means of enhancing the diversity of ectomycorrhizas in managed stands.

## Chapter One -- Diversity of ectomycorrhizal types

### Introduction

Each species of ectomycorrhizal fungus has unique environmental tolerances and physiological capabilities (e.g. Hutchison 1991) and hence a potentially unique value to its host. An ideal description of a community of ectomycorrhizal fungi would include the distribution and abundance of all species present. Only a small proportion of ectomycorrhizas have been described in detail and fewer still have been identified to species. Therefore a detailed survey of ectomycorrhizas must include a description of each morphological type encountered in sufficient detail to allow all types to be distinguished. Because techniques for identification of ectomycorrhizas are time-consuming and not always successful, most surveys deal primarily with unidentified types and probably do not detect many of the less common types. Types described in detail usually represent a single species of fungus or several closely related species (Dominik 1969, Godbout and Fortin 1985a, Zak 1973).

A comprehensive description of the features of an ectomycorrhiza, and attempted identification, require detailed microscopic examination (Agerer 1991, 1993) and take about two weeks to complete (Agerer R. 1995, personal communication). Ectomycorrhizas are most commonly identified by tracing rhizomorphs or mycelial strands that connect the ectomycorrhizas to sporocarps of known identity, or by comparing mycelium at the base of sporocarps to mycelium on ectomycorrhizas (Chilvers 1968, Ingleby et al. 1990, Zak 1971b, 1973). Without attached sporocarps, routine identification of most of the fungal symbionts of a field collection of ectomycorrhizas will not be feasible until many more descriptions and more extensive keys are made. Shorter lists of the most useful and easily ascertained characters that will allow one to separate a

limited number of types are given by Chilvers (1968), Dominik (1969), Ingleby et al. (1990) and Voiry (1981).

There are probably on the order of 500 naturally occurring ectomycorrhizas of Douglas-fir (Trappe 1977, Zak 1973). To date no comprehensive descriptions have been made of ectomycorrhizas of Douglas-fir, although the excellent works of Zak (1969, 1971a) and Zak and Larsen (1978) for *Byssoporia (Poria) terrestris* (DC. ex Fries) Larsen & Zak varieties and *Rhizopogon vinicolor* A.H. Smith are almost as detailed as present standards.

This study was designed to compare diversity of morphological types of ectomycorrhizas in old-growth and mature stands. Because identification of ectomycorrhizas was of lower priority, identification was attempted for only the most common or distinctive types. A subordinate objective was to characterize all types encountered with enough detail that most would likely represent a single fungal species or a few closely related species, in order that conclusions about types would likely apply also to species.

## Literature review -- Characterization and identification of ectomycorrhizas

**1. Introduction.** Feeder roots of Douglas-fir and other conifers are predominantly ectomycorrhizal, and roots bearing root hairs are less frequent (McMinn 1963, Meyer 1973, Roth 1990). Ectomycorrhizal roots are readily distinguished at low magnification (10X) by their swollen appearance and their fungal sheath or mantle. Root tips that have a rudimentary mantle or none may be poorly developed ectomycorrhizas, non-mycorrhizal, ectendomycorrhizal (Danielson 1982), or colonized by zygomycetous fungi of the genus *Endogone* (Chu-Chou and Grace 1979, Fassi and Palenzona 1969, Roth 1990). Mycorrhizas formed by *Endogone* are often considered to be ectomycorrhizas, but have a distinct internal structure (Roth 1990). Ectendomycorrhizas are formed by fungi in the genus *Wilcoxina* (Egger and Fortin 1990) or by other ascomycetes.

The identity of an ectomycorrhiza is precisely specified as the species of both the host plant and the fungal symbiont. In most cases, the plant is readily identified and contributes relatively little to the distinguishing features of the ectomycorrhiza; thus identification of ectomycorrhizas is largely a problem in mycology.

### **2. Taxonomic diversity of ectomycorrhizal fungi.**

Ectomycorrhizas are formed by a variety of fungi in the subphyla Basidiomycotina and Ascomycotina, of which some of the most common are listed in Table 1.1. The Polyporaceae are predominantly wood-rot fungi, but do contain some ectomycorrhizal species found in rotting wood, e.g. *Byssoporia* (Zak and Larsen 1978). Most families in the Agaricales and Gasteromycetes are either mycorrhizal or non-mycorrhizal. The Phallales are non-mycorrhizal except for *Hysterangium*, which contains some of the most common and abundant ectomycorrhizal fungi in native Douglas-fir forests (Griffiths *et al.* 1991b). Trappe (1971) listed ascomycete ectomycorrhizal species in 12 families and 22 genera,

including *Elaphomyces*, *Genea*, *Hydnotrya*, *Tuber*, *Helvella*, *Otidea*, *Geopora* and *Gyromitra*. A comprehensive list of species of ectomycorrhizal fungi and their hosts (Trappe 1962) included 51 ectomycorrhizas of Douglas-fir, although many of these were based on the constant association of sporocarps with the host, rather than actual identification.

Zak (1973) conservatively estimated that there are 100-200 ectomycorrhizas of Douglas-fir in the Pacific Northwest of the USA, whereas Trappe (1977) estimated that there are 2000 fungi with the potential to form ectomycorrhizas on Douglas-fir in nature. Surveys of ectomycorrhizas of exotic Douglas-fir forests in New Zealand found fewer than 30 species (Chu-Chou and Grace, 1981a, 1983a, 1987), although the surveys were not designed to measure diversity. Native Douglas-fir forests certainly contain a much greater diversity of ectomycorrhizas. Most fungi that produce hypogeous sporocarps are thought to be ectomycorrhizal (Hunt and Trappe 1987, Luoma et al. 1991, Miller 1983). Luoma et al. (1991) found 47 species of hypogeous fungi fruiting in 10 Douglas-fir stands of various ages in Oregon. The Tuberales, which produce hypogeous sporocarps known as true truffles, are ectomycorrhizal fungi, including the gourmet species *Tuber melanosporum* Vitt. and *Tirmania africana*.

**3. Characterization of ectomycorrhizas.** Zak (1971b, 1973) and more recently Agerer (1986a, 1986b, 1993) have listed features of ectomycorrhizas useful in classification, and described methods for their observation and description. If a description is to be published to aid others in the differentiation and identification of ectomycorrhizas, then as many characters as possible should be used (Agerer 1993, Chilvers 1968, Zak 1971b). Complete descriptions require much effort. Shorter lists of the most useful and easily ascertained characters that will allow one to separate a limited number of types are given by Ingleby et al. (1990), Voiry (1981), Chilvers (1968) and Dominik (1969). Important characters observable at low magnification (10-40X) are colour; surface texture; and shape and size of emanating hyphae, cystidia, mycelial strands and rhizomorphs. At high

magnification (400-1000X), important characters are shape, size and arrangement of hyphae or cells in the outer and inner layers of the mantle; presence of clamp connections; and features of emanating hyphae, strands or rhizomorphs. Anatomical features that can only be viewed with a compound microscope at 1000X need to be ascertained to recognize many ectomycorrhizas, although other more distinctive ectomycorrhizas may with experience be recognized at 40X magnification under a dissection microscope (Danielson 1982). Some of the more important or difficult characters warrant mention.

**a. Mantle structure.** Chilvers (1968) made a valuable contribution by clearly describing the types of mantle tissue structure as felt and net prosenchyma, and regular and irregular synenchyma. Prosenchyma (also called plectenchyma) is a network of hyphae containing interhyphal spaces, whereas synenchyma (also called pseudoparenchyma) has a compact cellular structure. With minor modification, these categories were clearly illustrated and described by Ingleby et al. (1990). Agerer (1993) recognized 16 types of mantle surface anatomy, by describing variation within the six groups of Ingleby et al. (1990), as well as combinations of these forms. Mantle structure can be observed in squash mounts of ectomycorrhizas, in pieces of the mantle scraped from the ectomycorrhiza, and in glancing sections. The inner and outer surfaces of the mantle generally have different structures. The thickness of the mantle has been considered too variable to be of much use (Chilvers 1968, Zak 1973), but was used by Roth (1990) in a key to 47 types of ectomycorrhizas on Douglas-fir seedlings.

**b. Setae and cystidia.** Setae (bristle-like) or cystidia (hair-like) are modified hyphae that have grown out from the mantle, and are often distinctive (e.g., Dominik 1969). These have been used to distinguish species of *Tuber* (see references in Voiry, 1981).

**c. Colour.** Many ectomycorrhizas have transparent or translucent mantles that are colourless; consequently their colour is largely that of the host's cortex (Chilvers 1968,

Godbout and Fortin 1985a). The outer cell layers of the cortex are usually compressed and contain tannins, forming a "tannin layer" (Chilvers 1968, Dominik, 1969), which is brown with yellow, red or orange tints. The colour of other ectomycorrhizas is entirely the result of yellow, brown, green or blue pigments in the mantles. White or silvery and reflective appearance of ectomycorrhizas is generally the result of air spaces within the mantle or of fine surface texture due to exudates or encrustation. Combinations of these three colour sources can occur. Colours often change with aging, e.g. *Lactarius deterrimus* Gröger turns from bright copper orange (host pigment) to dark olive (fungal pigment) with age (Agerer 1986a). Both tannin layer pigments and fungal pigments tend to darken as ectomycorrhizas age.

**d. Hyphal characters.** Hyphae in the mantle or emanating from it can be distinctive in shape, size, wall thickness, septation, surface deposits or ornamentation and shape of clamp connections. The presence of hyphal encrustations is a consistent and distinctive character on ectomycorrhizas (Agerer 1993), but not on hyphae in cultures of ectomycorrhizal fungi (Hutchison 1991). Within certain genera clamps are consistently absent, e.g. *Lactarius*, or rare, e.g. *Suillus*, *Tricholoma*, while others have clamps at every septum, e.g. *Hygrophorus*, *Laccaria*, *Hebeloma*, *Boletinus*, *Paxillus* and most species of *Cortinarius* (Hutchison, 1991). *Amanita muscaria* (Fr.) S.F. Gray has clamps in older parts of hyphae only. Nobles' (1971) rule, that if basidiocarps possess clamps so will dikaryotic cultures, can probably be extended to ectomycorrhizas, although it is difficult to determine whether an ectomycorrhiza is formed by a monokaryon or dikaryon if clamps are absent. Clamp connections may be distinctive in size and shape.

**e. Internal anatomical features.** Sections reveal useful features of mycorrhizal tissues and of fungal and host cells. Hartig nets vary in the extent of their penetration into the cortex (e.g. Roth 1990) and in appearance (Agerer

1993). *Leccinum* species are easily recognized on the basis of a peculiarly beaded appearance of Hartig net hyphae (Agerer 1993, Godbout and Fortin 1985b). Size and shape of tannin and cortical cells are other features seen in root cross-sections (Agerer 1987, Godbout and Fortin 1985a).

**f. Rhizomorphs and mycelial strands.** Some use the term rhizomorph only for well-differentiated mycelial strands that have smooth surfaces and inner and outer layers that are structurally and functionally distinct from one another. In this case the outer protective layer is often pigmented and the inner layer is composed of larger hyphae specialized for conduction. Others refer to all strands as rhizomorphs (as I shall) or, alternatively, as strands. According to Chilvers (1968) and Agerer (1986a) almost all mycorrhizas have rhizomorphs attached, but those on ectomycorrhizas with smooth mantles, such as those formed by *Russula* and *Lactarius*, are easily detached while cleaning the roots. In some cases where rhizomorphs have been reported lacking, it is likely that they have been inadvertently lost. The shape, size, branching pattern, colour and anatomy of rhizomorphs are important characters (Agerer 1986a, Agerer 1993, Chilvers 1968, Ingleby et al. 1990). Anatomical features of rhizomorphs include the size of vessel-like hyphae, nature of emanating hyphae and presence of a pigmented surface layer.

**g. Reactions of fungal tissue to chemical reagents.** Besides morphological and anatomical features, there may be reactions of tissues and hyphae of ectomycorrhizal fungi to various chemicals. Colour changes can occur and hyphal encrustations may be dissolved or changed. Like hyphal features, the results of chemical tests can be related to fungal taxonomy, aiding in identification. Reactions to chemical reagents can be observed at both low and high magnifications.

**h. Autofluorescence.** Ectomycorrhizas or tissue of ectomycorrhizal fungi may fluoresce various colours when exposed to long- or short- wave ultraviolet radiation. Whole ectomycorrhizas may fluoresce and tissues can be

examined with an epifluorescence microscope.

**4. Identification of ectomycorrhizas.** Chilvers (1968), Ingleby et al. (1990), and Zak (1971b, 1973) have discussed the methods for identification of ectomycorrhizas, which are preferably used in combination to reach a positive identification.

**i) Compare unknown ectomycorrhizas with ectomycorrhizas synthesized using cultures of fungi of known identity.** This method and others that require cultures of ectomycorrhizal fungi cannot help identify ectomycorrhizas formed by fungi for which methods of culture are unknown, such as most species of *Russula*, the Gomphidiaceae, many in Amanitaceae and some in Tricholomataceae and Cortinariaceae (e.g. *Inocybe*) (Agerer 1986b). These fungi are likely obligate symbionts with complex and specialized nutritional requirements. Moreover, most ectomycorrhizal fungi that can be cultured grow very slowly. If a fungus can be isolated from a sporocarp found near an unknown ectomycorrhiza, then it can be placed in monoxenic culture with roots of seedlings in an attempt to "synthesize" the ectomycorrhiza. Unfortunately, even with the same isolate, natural and synthesized ectomycorrhizas may differ in morphology, due to the presence of sugars in the culture medium and difference in age of the ectomycorrhizas (Godbout and Fortin 1985b). If sugars are not used in the growth medium, synthesized ectomycorrhizas should resemble young natural ectomycorrhizas (Godbout and Fortin 1985b). Nevertheless, Agerer (1987) recommends against the use of synthesized ectomycorrhizas for characterization or identification.

**ii) Compare cultures of fungi isolated from unknown ectomycorrhizas with cultures of known fungi.** Chu-Chou and Grace (1981a, 1981b, 1983a, 1983b) used this method successfully to identify numerous ectomycorrhizas in New Zealand conifer plantations. It is best to use isolates from sporocarps found in the vicinity of the ectomycorrhiza rather than isolates in culture collections or herbaria. Hutchison's (1991) key separated 95 species of ectomycorrhizal fungi in pure culture on the basis of their

ability to degrade carbon and nitrogen compounds, tolerance to temperature extremes and temperature preferences, polyphenol oxidase activity, staining with diazonium-blue-B and colony morphology. Besides the difficulty in culturing ectomycorrhizal fungi, other problems with this method are i) cultures can spontaneously change from dikaryons to monokaryons after a few transfers, in which case clamp connections are lost and other cultural characters may change (Hutchison 1991); and, ii) different fungi can look similar in culture (Agerer 1986b). Dominik (1969) considered that cultural characters are of limited value for ecological studies. Chilvers (1968) considered isolation and comparison of cultures to be time-consuming and not often successful, although of some use in relating surveys of ectomycorrhizas and sporocarps.

**iii) Compare the mycelium and rhizomorphs attached to unknown ectomycorrhizas with those attached to the base of nearby sporocarps of known identity.** This was Zak's (1973) favourite method, because he found it accurate, reliable, readily applied and it did not require culturing. But Agerer (1986b) thinks it provides insufficient evidence in forests with many fungi in the soil.

**iv) From unknown ectomycorrhizas, trace hyphae or rhizomorphs that form a continuous connection to a sporocarp of known identity.** This method may be tedious, inaccurate if not carefully done, and may be impractical if ectomycorrhizas and sporocarps are far apart (Agerer 1986b, Chilvers 1968), but has been used extensively by Agerer (1986b). His method of demonstrating connections is to remove the sporocarp and an underlying core of soil with a sharp knife, cut the stipe (stalk of the sporocarp), soak in water the soil and base of the stipe, then, with the sample immersed, carefully and gradually wash the soil away from the stipe and any ectomycorrhizas, using very fine needles, forceps, paint-brushes or pipets. If done "critically and patiently", Agerer (1986b) considers this a good method, especially if there are rhizomorphs.

**v) Compare unknown ectomycorrhizas with published**

**descriptions of identified ectomycorrhizas, possibly with the assistance of keys.** This is not yet feasible except for the most distinctive and common of ectomycorrhizas, as insufficient detailed descriptions have been published. However, the colour guides of Agerer (1993) and Ingleby et al. (1990) allow rapid comparison with about 70 identified ectomycorrhizas of Europe.

A sixth method, currently being developed, compares DNA "fingerprints" of fungi of unknown ectomycorrhizas with a "library" of DNA fingerprints from known fungi (e.g. Gardes et al. 1991).

In most cases identification of the fungal symbiont of a field collected ectomycorrhiza is impractical (Hutchison, 1991) and will remain so until many more descriptions and more extensive keys are made. Current keys cover only subsets of the symbionts present in a limited area (Agerer 1993, Roth 1990). Roth's (1990) key to species found on conifer seedlings outplanted on Vancouver Island uses staining reactions, mantle thickness, distance between septa, features of the Hartig net, mantle structure, morphology of setae and cystidia and other characters. Agerer's (1993) key uses rhizomorph anatomy, colours of the ectomycorrhizas, mantle anatomy, setae and cystidia. Comprehensive keys have long been envisioned by ecologists, who need identifications to relate the results of one study to another and to get information on the ecology of the fungi being studied (Pentland 1959, Zak 1973, Roth 1990). At present however, description of ectomycorrhizas without identification of the fungus is sufficient for many field studies and can contribute to the development of keys, especially if type specimens are preserved (Zak 1971b). It seems likely that comprehensive keys will eventually be developed because ectomycorrhizal morphology and anatomy is fairly constant despite environmental variation (Zak 1973). Of more concern are subspecific variation and differences between monokaryons and dikaryons of the same species. Variation such as that of *Rhizopogon vinicolor*, which forms monopodial pinnate ectomycorrhizas or tuberculate

ectomycorrhizas on seedlings and young trees, but forms only tuberculate ectomycorrhizas on older trees (Chu-Chou and Grace 1981a), may also cause difficulties.

A single key should suffice for several hosts in the same family, as similar ectomycorrhizas are formed by the same fungus on different hosts (Godbout and Fortin 1985a, Ingleby et al. 1990, Molina and Trappe 1982). However, Molina and Trappe (1982) found some differences in synthesized ectomycorrhizas due to the host. More tannins (tannins and other phenolic substances toxic to fungi are often formed in response to infection) were formed in the cortex of some hosts than others, indicating a different host-symbiont interaction. And in some cases there were minor differences in the compactness of the mantle and abundance of rhizomorphs. The branching pattern of systems of ectomycorrhizas is a host-determined feature (Godbout and Fortin 1985a, Molina and Trappe 1982, Zak 1973), but the degree of branching is determined by the fungus (Godbout and Fortin 1985a).

Morphology and anatomy can identify genera of ectomycorrhizal fungi, but will not separate all species (Dominik 1969, Godbout and Fortin 1985a, Zak 1973). Zak (1973) found pairs of species in *Lactarius* and in *Cortinarius* that had indistinguishable ectomycorrhizas. He suspected that ectomycorrhizas of *Lactarius deliciosus* and *L. sanguifluus* Fr. would also be identical. Godbout and Fortin (1985a) found that types of structure of the mantle of ectomycorrhizas of *Amanita* corresponded to subdivisions of the genus. They suggested that it may not be possible to separate *Amanita* species on the basis of ectomycorrhizal morphology and anatomy. They concluded that chemical techniques may be essential to identify ectomycorrhizas to the species level.

Keys to ectomycorrhizas can probably be taxonomically organized, as morphological groupings of ectomycorrhizas confirm fungal taxonomy (Ingleby et al. 1990, Voiry 1981). Ectomycorrhizas in the same genus have similar characteristics and some closely related genera have similar

morphology (Godbout and Fortin 1985a, Voiry 1981). Thus *Leccinum* has beaded hyphae in the Hartig net, Cortinariaceae has woolly ectomycorrhizas, the Russulaceae are smooth, *Lactarius* has laticiferous hyphae and *Russula* has cystidia. Voiry (1981) divided ectomycorrhizas into three main groups as did early workers (see reference in Agerer 1986a): group A with prosenchymatous outer mantles and abundant emanating hyphae or poorly differentiated rhizomorphs, group B with synenchymatous mantles and rhizomorphs and group C with smooth synenchymatous mantles and no emanating hyphae or rhizomorphs. As mentioned above, it may be that species in group C do have well-differentiated rhizomorphs that are infrequent and easily detached. Some related taxa shared the same groupings: *Hebeloma* and *Cortinarius* in A, Boletaceae and Sclerodermataceae in B and *Lactarius* and *Russula* in C. Genera tend to be placed along a "morphostructural series" (Godbout and Fortin 1985a), meaning simply that the more compact the mantle structure, the fewer emanating hyphae or rhizomorphs are present. Ascomycetes have compact mantles but can only be differentiated from basidiomycetes by hyphal features. Some basidiomycetes are readily recognized as such by the presence of clamp connections. If clamps are absent, the class of an ectomycorrhizal fungus can sometimes be determined by the more difficult task of examining hyphal septa for dolipores (basidiomycetes) or Woronin bodies (ascomycetes), staining nuclei (Agerer 1986b), or isolating the fungus in axenic culture and testing for sensitivity to the fungicide Benomyl (ascomycetes are sensitive, basidiomycetes are not) (Agerer 1986b, Danielson 1982).

**5. Descriptions of ectomycorrhizas.** The *Colour atlas of ectomycorrhizas* (Agerer 1993) and *Identification of ectomycorrhizas* (Ingleby et al. 1990) show colour photos of about 70 identified ectomycorrhizas of a variety of basidiomycetes and ascomycetes on *Picea abies* (L.) Karst. and *Larix decidua* Mill. in Europe and on *P. sitchensis* (Bong.) Carrière and *Betula pendula* Roth in Britain, respectively, with photos of mantle structure, hyphae and

rhizomorphs. Each ectomycorrhiza in the Colour Atlas is further described in a cited journal article. The ectomycorrhizas of *Tricholoma*, *Lactarius* and *Russula* were reviewed by Agerer (1987, 1986a).

Voiry (1981) described 13 ectomycorrhizas of oak and beech in north-eastern France. Chu-Chou and Grace (1983a) described 12 types of Douglas-fir ectomycorrhizas in New Zealand, of which 8 were identified: *Rhizopogon* sp., *Rhizopogon vinicolor*, *Tuber*, *Amanita muscaria*, *Hebeloma crustuliniforme* (Bull. ex St. Amans) Quél., *Laccaria laccata* (Scop. ex Fr.) Cke., *Thelephora terrestris* and *Boletus*. Chilvers (1968) described eight unidentified ectomycorrhizas on *Eucalyptus*. Other articles in numerous journals describe ectomycorrhizas, both natural and synthesized.

In a series of studies, natural ectomycorrhizas of *Byssoporia terrestris* vars. *sartoryi*, *aurantiaca*, *lilacinorosea*, *parksii* and *sublutea*, and *Rhizopogon vinicolor* on Douglas-fir in Oregon were described in detail (Zak 1969, 1971a, Zak and Larsen 1978). Molina and Trappe (1982) described ectomycorrhizas on seven conifers of the Pacific Northwest of the United States synthesized with 27 fungi. Trappe (1967) described synthesized ectomycorrhizas of *Hebeloma*, *Suillus*, *Rhizopogon* and *Astraeus* on Douglas-fir. Froidevaux (1975) described ectomycorrhizas of *Cantharellus cibarius* Fr., *Piloderma byssinum* (Karst.) Juelich (this is not *P. byssinum* (Danielson R.M. 1995, personal communication)) and *Inocybe geophylla* (Sow. ex Fr.) Kumm. on Douglas-fir in Oregon. Hunt (1991) produced a colour guide to the most common ectomycorrhizas in container nurseries in British Columbia. Danielson et al. (1984) described ectomycorrhizas of *Tomentella* Pat., *Amphinema byssoides* (Pers.: Fr.) Erikss. and a *Rhizopogon*-like ectomycorrhiza on spruce in Alberta. Roth (1990) described 44 types on outplanted conifer seedlings on Vancouver Island, including colour plates of *Thelephora terrestris* (Ehrh.) Fr., *Rhizopogon vinicolor*, *Cenococcum geophilum*, *mycelium radicus atrovirens* (mra) and *Tuber*-like ectomycorrhizas.

Some plant-fungus combinations of synthesized ectomycorrhizas may not exist in nature (Zak 1971b). Yet the ability of a fungus to form a synthetic ectomycorrhiza with a particular host is evidence that it may do so in nature if the fungus fruits frequently in association with that host (Chilvers 1968).

**6. Conclusions.** Progress in describing European ectomycorrhizas continues to be made with additions to the *Colour Atlas* (Agerer 1993), but the number of fungal symbionts and the time required to make complete characterizations has discouraged new descriptions of ectomycorrhizas of Douglas-fir in British Columbia and the Pacific Northwest. European descriptions are useful at the generic level but are not sufficient for use with ectomycorrhizas from the west coast of North America because there are important differences in the fungal flora of the two regions. Two of the most common ectomycorrhizas of mature Douglas-fir in British Columbia have been well described, ie. *Rhizopogon vinicolor* (Luoma et al. 1991, Zak 1971a) and *Cenococcum geophilum* (Ingleby et al. 1990, Roth 1990, Wilcox and Wang 1987), but dominant fungi such as *Hysterangium crassirhachis* Zeller & Dodge (Luoma et al. 1991) have not been described. There is now an opportunity to describe important ectomycorrhizas in this region, which might stimulate and facilitate research into the ecology of ectomycorrhizas.

## Methods and materials

**1. Sampling and storage of soil.** A cylindrical, steel coring tool was used to collect samples 5 cm in diameter by  $\leq 15$  cm in depth. Core samples of soil were taken to a depth of 15 cm from the surface of the forest floor unless the tool was blocked by rock or roots. Samples were not taken deeper than 15 cm because previous studies indicated that most ectomycorrhizas in Douglas-fir stands occur in the forest floor and in the uppermost few centimetres of mineral soil. To reduce disturbance of the soil and mycorrhizas, the tool was kept sharp, was inserted slowly with a twisting motion, and the soil cores were stored intact, as sampled, in acrylic tubes that slid into and out of the coring tool. The ends of the tubes were closed with plastic caps. Within 8 hr of sampling, soil samples were put into storage at 2 C in the dark, in closed but unsealed plastic bags that contained some water to prevent drying of the soil. Soil samples were stored for  $\leq 120$  days before extracting ectomycorrhizas. Most samples were stored fewer than 50 days.

**2. Extraction of ectomycorrhizas from soil.** Soil was pushed from the storage tubes with a cylindrical container slightly smaller in diameter than the inside of the tubes. Each soil core was cut in half longitudinally with a sharp knife. Roots were extracted from one half, and the other was analyzed for nutrients. The litter, fragmented litter, and humus layers of the forest floor (the three layers of the surface organic layer are hereafter called LFH), were processed separately from mineral soil and processed separately. Soil was washed in a 0.5 mm sieve with distilled water while clumps of root-bound soil and decayed wood or bark were cut or broken into pieces smaller than 4 cm diameter. Distilled water was used because chlorine or other compounds in tap water may have affected the morphology of the ectomycorrhizas. Washing was thorough and with enough water pressure that soil particles less than 0.5 mm were mostly removed. Care was taken to minimize friction

of ectomycorrhizas against the sieve. Roots in large (> 1 cm) clumps of wood, bark or tightly bound soil were dissected in distilled water in Petri dishes under a Wild M3Z dissecting microscope at 6X. Higher magnifications were occasionally used to look for rhizomorphs, trace roots through the soil, and determine if mycorrhizas were living. At all times during examination, soil and ectomycorrhizas were kept in distilled water. Smaller fragments of roots and soil were evenly spread in a 23 cm by 36 cm glass pan in a layer of water thin enough that both the top and bottom of the water could be in focus at once. The pan was then scanned at 6X and all ectomycorrhizas were removed. Roots without a mantle or with thin, loosely organized mantles were usually left in the pan. Occasionally, roots with rudimentary mantles that had the swollen appearance and branching pattern characteristic of ectomycorrhizas were extracted and examined. No attempt was made to distinguish host species of mycorrhizas. Ectomycorrhizas were extracted from the soil and cleaned using a pair of fine forceps in each hand. A fine paintbrush, a fine insect pin and a small water bottle were also used in cleaning ectomycorrhizas sufficiently to observe their texture, colour, rhizomorphs, branching pattern and other morphological features.

**3. Separation of types within a soil sample.** The ectomycorrhizas extracted from the LFH or mineral layers of a core sample were separated into groups based on their morphology as examined at 6X-40X under the dissecting scope. Mantles from two or more tips from each group were examined at 1000X with a Leitz Labovert compound microscope, to confirm that the ectomycorrhizal tips within a group were sufficiently similar to be included in the same type. Fewer slide mounts were made from types that were easily recognized under the dissecting scope.

**4. Description of types of ectomycorrhizas.** Types represented by five or fewer tips in a core sample were not described. The features of ectomycorrhizas that were examined are listed in Table 1.1. Several chemical reagents were applied to whole ectomycorrhizas to check for colour

reactions. The chemical basis of most of these reactions is not known. Melzer's reagent is known to produce red (dextrinoid) or blue (amyloid) reactions due to the effect of iodine on dextrin and amylose or similar compounds (Baral 1987). Fragments of the mantle were scraped or peeled from the roots at 40X using a fine (number 0) insect pin mounted on a wooden stick. Six to twelve fragments were arranged on a slide with half of them upside-down, as indicated by curvature or the presence of some host cells. Beseler 50 Cyan 1.8X and 10 Magenta 1.2X filters were used to convert the microscope light sources to daylight quality for all observations. Photographs of the anatomy of the inner and outer mantle, rhizomorphs, emanating hyphae and cystidia, were taken at 1000X using a Wild MPS 12 camera and a Wild MPS 05 exposure meter. Whole ectomycorrhizas were photographed at 6.5-40X in water, above a dark background except for dark ectomycorrhizas, which were photographed above a light background. Fujichrome professional RTP 135 tungsten film was exposed with unfiltered light. The following steps prepared ectomycorrhizas for scanning electron microscopy: root tips were fixed in formalin-acetic acid for 24 hr, stored in 70% ethanol (EtOH) for up to 4 months, sectioned if necessary; dehydrated with immersion for 1 hour in each of: 80% EtOH, 95% EtOH, absolute EtOH, 50% EtOH and 50% acetone, fresh acetone and fresh acetone; critical point dried from CO<sub>2</sub> and coated with gold/palladium.

**5. Estimating richness of types.** Plots of the number of types observed as a function of the number of observations of types (an observation is defined as its occurrence in a core sample) were used to examine the rate that new types were being found. With sufficient sampling these curves would approach an asymptote that is at the level of the total number of types in the area sampled. Plots of abundance of types (logarithms of the number of root tips colonized or number of soil cores colonized) versus the types' rank according to such abundance were used to compare equitability (the degree to which types are equally

abundant) and richness (the total number of types present) (Southwood 1978). On a log(abundance) versus rank plot total richness is equal to the x-intercept if the plot is linear.

## Results

### 1. Diversity of morphological types of ectomycorrhizas.

Sixty-nine morphological types of ectomycorrhizas were described. A few types (at most six) may not have been described because they did not colonize more than five tips in any sample. Nineteen types each accounted for more than one percent of the 17,500 ectomycorrhizal root tips examined. Eighteen types were found in five or more of the 120 soil cores. Each of the six most common ectomycorrhizas accounted for between 3 and 24 percent of the total number of root tips and occurred in 14-75 percent of the soil cores (Fig. 1.1). More than 97 percent of live root tips had a well-developed fungal mantle, excluding roots that were obviously not those of Douglas-fir. Live root tips were recognized by their strong, light-coloured stele. Those root tips without a well-developed mantle were not examined in detail because they were not readily identifiable as ectomycorrhizas. Equitability curves (Fig. 1.2) were similar in shape to the curve that results when the distribution of species in abundance classes is lognormal. Linear fits of the curves of Figure 1.2 result in lines which approximate the result of log series distributions, with diversity indices of  $\alpha=14$  (see Southwood 1978). A plot of the number of types described as a function of the number of observations of ectomycorrhizas (an observation is defined as the occurrence of a distinct type within a soil core) shows a decline in the rate of discovery of types not previously encountered in this study, although it is not clear where an asymptote might lie (Fig. 1.3). Figures 1.2 and 1.3 suggest a total richness of perhaps 100 types.

A database of all features of all types is listed in full in Appendix 1A, and a dichotomous key (Appendix 1B) was compiled for all types. The degree of confidence with which each type can be said to be formed by a distinct fungal species was gauged, resulting in three classes. Appendix 1C contains synopses of the descriptions of the 30 types that were either distinctive or were common and had

consistent features (class a, 14,900 tips). These types and their most distinctive features are illustrated in Figures 1.4-1.13. Twenty-seven types (class b, 2,300 tips) were either not distinctive, or were variable or were based on few observations, but would likely be reencountered and clarified with continued sampling. The descriptions of 11 types (class c, 260 tips) are not likely to be of use because they lack clarity or detail, or because they were based on only a few root tips or on ectomycorrhizas that appeared senescent. Voucher specimens of all types of ectomycorrhizas were kept in 10% glycerol in liquid nitrogen.

**2. Identity of the ectomycorrhizas.** The host plant species were not determined, although Douglas-fir dominated in all stands studied. In several cases, mycosymbionts were recognizable. *Cenococcum geophilum* Fr., denoted em1 (ectomycorrhiza number one), was both the most abundant and the most commonly encountered type (Figs. 1.1, 1.7a-c). Each type was given an overall or combined rank equal to the average of its rank by number of root tips colonized and its rank by number of soil cores in which it was observed. In second place was a white prosenchymatous type with abundant loose rhizomorphs and elongated crystals on its hyphae, em2 (Figs. 1.3, 1.7d-h), that formed mats of soil, rhizomorphs, mycelium, and ectomycorrhizas resembling mycorrhizas formed by *Hysterangium* species (Luoma D.L. 1992, personal communication). Type 2 also resembles *Piloderma byssinum* (P. Karst.) Jülich (Danielson R.M. 1995, personal communication). Third, em7 (Figs. 1.3, 1.4d, 1.8h, 1.9a, 1.9b), was *Rhizopogon vinicolor* A.H. Smith or a similar species in the section *villosuli*.

*Lactarius deliciosus* (Fr.) S.F.G., which formed the fourth most common type (Fig. 1.3), em13, was identified by the presence of laticiferous hyphae containing orange latex (Fig. 1.5b), and green colour reaction to age or bruising (Fig. 1.5a). The fifth ranked type, em3, (Fig. 1.3) was *Piloderma fallax* (Libert) Stalpers (Stalpers 1984) (= *Piloderma bicolor* (Peck) Jülich *sensu* Jülich = *Piloderma*

*croceum* J. Erikss. & Hjortstam.) considering its bright yellow colour (Fig. 1.4a) (there are no other golden species of *Piloderma* in North America (Danielson R.M. 1995, personal communication), ornamented clampless hyphae (Fig. 1.8a, b) and similarity to the description *P. croceum* on *Fagus sylvatica* (Agerer 1993). Sixth, em27 (Figs. 1.3, 1.11g, 1.11h, 1.12a), was similar to a mycorrhiza linked to a sporocarp of *Amanita muscaria* (Fr.) S.F. Gray var. *formosa*.

*Gomphidius* sporocarps were also linked to mycorrhizas. An ectomycorrhiza (probably of *Alnus rubra* Bong.) similar to that of em18 was linked to *Gomphidius glutinosus* (Schaeff: Fr.) Fries and an ectomycorrhiza similar to em58 and em60 was linked to a sporocarp similar to *Gomphidius smithii* (Schaeff. ex Fr.) Fries.

Type 68 closely resembles *Hydnellum peckii* Banker apud Peck + *Picea abies* (L.) Karst as described by Agerer (1993) and is similar to an ectomycorrhiza linked to a member of the Hydnaceae. Other types corresponding with descriptions in Agerer (1993) are em20 (Fig. 1.5d) with *Amphinema byssoides* (Pers.) J. Erikss. + *Picea abies* (L.) Karst., em28 with "*Fagirhiza spinulosa*" (unidentified + *Fagus sylvatica* L.), em41 with "*Piceirhiza bicolorata*" (unidentified + *Picea abies*) and em62 (Fig. 1.13h) with *Russula illota* Romagn. + *Fagus sylvatica* L.. The names "*Fagirhiza spinulosa*" and "*Piceirhiza bicolorata*" do not designate official taxa of fungi, but are names of ectomycorrhizas. Danielson doubts that em20 is *Amphinema*, considering its colour, lack of ornamentation on the hyphae, and lack of positive reaction to KOH (Danielson R.M. 1995, personal communication). The description of *Russula aeruginea* Lindblad:Fr. synthesized with *Picea sitchensis* (Bong.) Carrière (Taylor and Alexander 1989) is also very similar to em62. The *Russula* forming em62 is more likely to be *R. aeruginea* than *R. illota*, as the former species fruits more commonly in western North America.

Type 5 (Figs. 1.4b, 1.8d, 1.8e) has hyphae on the surface of its rhizomorph that are similar to those of *Byssoporia terrestris* (DC. ex Fries) Larsen & Zak (Zak and

Larsen 1978). Type 62 (*Russula*) and type 30 are similar to descriptions by Roth (1990) (Roth's types 13 and 33 respectively) of ectomycorrhizas on seedlings of Douglas-fir on southeastern Vancouver Island. Type 44 is probably a *Tuber* species, considering its long, tapered cystidia (Agerer 1993, Roth 1990). Types 14, 28, and 31 are *Tomentella*-like (Danielson R.M. 1995, personal communication).

**3. Diversity of characters used to distinguish ectomycorrhizas.** The primary characters used to distinguish ectomycorrhizas were colour, texture, presence and morphology of rhizomorphs, anatomy of the outer surface of the mantle, presence of clamp connections, presence and shape of setae or cystidia, presence and nature of hyphal ornamentation, size of hyphae and size of cells in the mantle. Other important characters less commonly used were presence of laticiferous hyphae, fluorescence under UV light and colour reactions to chemical reagents. The shape of root tips and the anatomy of the inner surface of the mantle were occasionally used.

All ectomycorrhizas in a soil sample were separated into homogeneous groups based on colour, texture and the morphology of rhizomorphs. Rarely did anatomical observations show two groups in a sample to be of the same type, or a single group to contain tips of more than one type.

**a. Colour.** Twenty-eight types were white or nearly so (e.g. Figs. 1.4b, 1.6c, 1.7d, 1.8d, 1.9c, 1.11a), 28 were brown (e.g. Figs. 1.5a, 1.5c, 1.6d, 1.10c, 1.12e, 1.13d) or light-yellow or light-orange (e.g. Fig. 1.11c), 7 were dark brown (e.g. Figs. 1.9f, 1.12b), 3 were distinctly yellow (Figs. 1.4a, 1.5d, 1.6a), 2 were black and one was distinctly orange. Several types that were essentially brown or white showed patches of colour or colour in response to bruising. Type 63 was white with orange, em5 was white with blue (Fig. 1.4b), em21 was brown with blue; and em13, *Lactarius deliciosus*, is brown with green (Fig. 1.5a). One "white" type was light-pink (em46) (Fig. 1.6c).

Whiteness was due to air trapped by the hyphae of a prosenchymatous mantle (e.g. Fig. 1.7d) or to cystidia on the surface of a synenchymatous mantle (e.g. Fig. 1.12e). I had some problems recognizing and placing in the key a few types that were both brown and white. Type 30 was white with brown patches due to the presence of dark brown exudates. Types 32 and 50 were brown, but with extensive white areas due to warts or hyphal growth. Types 31 (Fig. 1.12e) and 62 (Fig. 1.13h) varied from mostly white to all brown depending on whether their cystidia trapped air. Some white types with thin mantles became brown due to age or rubbing on the mantle. Type 14 was light brown when young (Fig. 1.5c), becoming dark brown with age.

**b. Texture.** The most common ectomycorrhizal texture was "reticulate" (26 types) (e.g. Fig. 1.6c), reflecting a prosenchymatous mantle. Smooth ectomycorrhizas (14 types) (e.g. Figs. 1.4c, 1.5a, 1.11c, 1.13d) were either synenchymatous (9 types) (e.g. Figs. 1.11d, 1.13e) or prosenchymatous and smooth due to an apparent gel-like matrix (types 4,6,24,38) (e.g. Figs. 1.8f, 1.8g) or due to a dense arrangement of narrow hyphae (type 41). Smooth types were either shiny (e.g. Figs. 1.4c, 1.5a, 1.13d), or matte (finely grainy) (e.g. Fig. 1.11c). Fourteen types appeared grainy. Graininess of prosenchymatous types was due to a variety of structures: cystidia (types 54, 62), branched hyphae (em5) (Fig. 1.4b), a dense network of thick hyphae (em9) (Fig. 1.9f), warts (em12) (Fig. 1.10c, 1.10d), a fine network (em19) (Fig. 1.11a), and bodies or small particles of mineral soil in the mantle (types 29,39,52,62) (e.g. Fig. 1.6a).

Six synenchymatous types were grainy, with setae (types 14,42) (e.g. Fig. 1.5c) or without (types 25,32,59,69). The grainy synenchymatous types (with the exception of em14, which had setae) were close to a smooth, matte appearance. Five synenchymatous types were spiny due to obvious cystidia (e.g. Figs. 1.12b, 1.12d, 1.12e). In addition to reticulate and grainy textures, prosenchymatous types were also stringy (4 types), woolly (3 types) and cottony (1 type), according

to the size and arrangement of hyphae.

**c. Reaction to chemical reagents.** Five types reacted to chemical reagents. Potassium hydroxide turned the mantle of em30 purple and the rhizomorphs of em46 blue. Meltzer's reagent turned the mantle and rhizomorphs of em10 dark and caused a blue-green colour in em59. Sulfovanillin turned em5 purple and em59 reddish purple. Ethanol, FeSO<sub>4</sub> and lactic acid produced no colour reactions. In each case of a colour reaction, the type was otherwise distinctive: types 10 and 46 were the only fluorescent types, type 46 was immediately recognizable by its pinkish colour (Figs. 1.6c, 1.13c) and had ornamented hyphae (Fig. 1.13c), type 5 had blue bruising (Fig. 1.4b), type 30 had dark globular exudates and type 59 had clear "plates" covering its surface (Fig. 1.13f).

**d. Anatomy of outer mantle and abundance of rhizomorphs.** Although each type was given a single number denoting the anatomy of its outer mantle, there was often variation within a type, or even within a root tip, that spanned two to three classes. In such cases the number recorded was that of the most common mantle type, usually the most highly differentiated. Types of outer mantle formed a continuum. Many types had outer mantles with an arrangement of hyphae or cells intermediate between two of the six classes of Ingleby *et al.* (1990). The outer layer of the mantle was prosenchymatous in 47 types, compared to 22 synenchymatous types. Prosenchymatous mantles were evenly split between felt prosenchymas (22) (e.g. Figs. 1.7e, 1.7g) and net prosenchymas (25) (e.g. Figs. 1.8e-g, 1.9b, 1.9d, 1.11e).

Synenchymatous mantles were also evenly distributed amongst the 4 classes used: 5 net (e.g. Figs. 1.7a, 1.13a), 5 interlocking (e.g. Figs. 1.11f, 1.13e), 8 with roundish cells (non-interlocking irregular synenchyma) (e.g. Figs. 1.11b, 1.11d, 1.12g) and 5 with polygonal cells (regular synenchyma) (e.g. Figs. 1.11d, 1.12c). Figure 1.14 shows the relationship between the structure of the outer mantle and abundance of rhizomorphs. Rhizomorphs were abundant only in prosenchymatous mantles (e.g. Figs. 1.4a, 1.5d,

1.6c, 1.7d, 1.10b), rhizomorphs were occasionally found in one type with a net synenchyma (em13, Fig. 1.5a) and in one type with an interlocking synenchyma, and no rhizomorphs were observed on any of the 13 types with synenchymas of roundish or polygonal cells. While an abundance of rhizomorphs indicates a prosenchymatous mantle, the converse is not true--many prosenchymatous mantles were observed without rhizomorphs attached (e.g. em 65, Fig. 1.6d).

The innermost layer of the mantle was almost exclusively a net synenchyma (51 types) (e.g. Fig. 1.7c, 1.10a), sometimes a net prosenchyma (14 types) (e.g. Fig. 1.8c) and rarely a felt prosenchyma (1 type) or interlocking synenchyma (1 type). There were two predominant types of net synenchymas in the inner mantles. Most common was an arrangement of cells that were less than 40  $\mu\text{m}$  long and were branched or bent. The other type of arrangement was one of layered groups of parallel hyphae, with the hyphae of different groups perpendicular to one another or sometimes at narrower angles, similar to the net prosenchyma of Fig. 1.8c. If present, layers between the inner and outer layers were anatomically like one or the other, although a few types showed some transitional anatomy in the middle layers.

**e. Clamp connections.** Clamp connections (e.g. Figs. 1.9d, 1.9e, 1.9g, 1.9h, 1.11g, 1.12e, 1.13b) were observed in 22 types, with some variation in clamp size and shape. Septa were observed in all types, although in several cases it was not clear whether the hyphae observed were of the same fungus as that forming the ectomycorrhizas.

**f. Cystidia or setae.** Cystidia or setae, present on 15 types, were useful for distinguishing types because they varied in size, shape, septation, ornamentation, pigmentation and thickness of walls. In 12 types the cystidia or setae were numerous and obvious (e.g. Figs. 1.12d, 1.12f, 1.12h, 1.13h) compared to three prosenchymatous types with variable, cystidia-like hyphal branches. Cystidia on synenchymatous mantles were more highly differentiated.

## Discussion

The predominance of ectomycorrhizas with well-developed mantles over other types of mycorrhizas was expected considering previous reports (Meyer 1973, Roth 1990). The six most abundant ectomycorrhizal fungi represented five orders, largely Basidiomycetous: Phallales (*Hysterangium*), Agaricales (*Lactarius*, *Amanita*), Hymenogastrales (*Rhizopogon*, related to Agaricales), Aphyllophorales (*Piloderma*) and (probably) an order in the Ascomycotina (*Cenococcum*). Richness of types, estimated at 100, was similar to other reports: Miller (1983) found 78 species of ectomycorrhizal fungi fruiting in an old-growth stand of western white pine, Luoma et al. (1991) found 47 species of truffle-forming ectomycorrhizal fungi fruiting in Douglas-fir stands and Zak (1973) estimated there are in total 100-200 ectomycorrhizas of Douglas-fir in the Pacific Northwest of the United States.

Description of each type was made from tips in the first sample in which the type was encountered, even if < 6 tips were present. Additional notes and measurements along with a sample number were added to the type description when a type was encountered again. In a study of shorter duration, it might be more efficient to separate types after they have been collected from a number of samples. This would allow more side-by-side comparisons of similar types. A larger group of tips would display a wider range of the morphological variation that results from variation in soil environment and aging of ectomycorrhizas. Groups of tips were found that could not easily be distinguished from previously found types, nor easily recognized as one of them. Side-by-side comparison would ease this problem, which arose most often in some white prosenchymatous types. The final group of white prosenchymatous types was not readily partitioned. Ectomycorrhizas can be frozen for later examination with minimal changes. Features such as reaction to chemical reagents, and perhaps colour, could be noted with fresh material prior to freezing. There is,

however, a limit to the amount of types and tips that can be compared at once, as morphological changes occur after several days storage in water.

Although subtle differences in the morphology of types may be difficult to describe precisely, they can be consistent. Given a hundred or so tips of varying ages, a type could with sufficient practice probably be distinguished by comparing morphology at 6X-40X under the dissection microscope with an extensive collection of photographs. It was possible to separate up to 10 types based on morphology when they were examined side-by-side.

To be used to distinguish morphological types of ectomycorrhizas, characters that are measured or described must be consistent within a type, or vary consistently within a limited range. Nearly all the characters selected for this study helped distinguish types, but most required some experience to assess accurately. Some quantitative morphological characters such as the diameter of tips were too variable to be readily useful. With many more observations, types might be found to have different diameter tips, but tip diameter is unlikely to be needed to distinguish types. Tip length, although not recorded, seems more likely to be useful. Qualitative characters were more easily used than the quantitative morphological characters recorded. Tip shape could usually be considered as bent, but there were many examples along a continuum between straight and tortuous. Tip shape varied with the substrate, for example, tips growing in mineral soil had more bends. Nevertheless, this character was also a function of the fungus and could be used to distinguish types with more careful observation and recording.

Colour varied greatly within many types and was misleading if only a few tips were observed, but its range within a type was consistent. Colour charts are useful for precise descriptions, but were too slow for routine use in this study. I prefer to describe the colour of an ectomycorrhiza as a combinations of colours if necessary, rather than using terms such as turquoise or lavender, which

are less easily understood (e.g. instead of turquoise I prefer bluish green). Colour was either that of the host surface in cases of transparent mantles, or that of the mantle, due to trapped air, surface texture or fungal pigment. Brown flecking of host cortex colour through a thin white prosenchymatous mantle was a consistent feature of some types. Rather than simply noting if the host cortex is visible, the amount in percentage of host colour showing and its distribution pattern could be recorded. Like colour, the textural appearance of the mantle surface varies due to age within a consistent range. Additional categories of surface texture are necessary in addition to the eight described by Agerer (1993). For example, mantles were found that were finely woolly or felty, smooth mantles were shiny or matte and there were different types of "reticulate" prosenchymatous mantles. Work is needed to precisely describe these textures, their relationships and boundaries.

Structure and branching pattern of rhizomorphs were consistent within types, and rhizomorphs of different types had different appearances under the dissection microscope, albeit subtly in some cases. At 40X under the dissection microscope a rhizomorph is easily classified as loosely organized or more densely constructed. Loose rhizomorphs were weaker and were usually flat with abundant branches at narrow angles ( $\leq 30$  degrees). Denser rhizomorphs were stronger, round in cross-section and usually without abundant branches.

Colour reactions to chemical reagents did not prove important because they were rare and occurred on otherwise distinctive types. I suspect the colour reactions are due to compounds involved in the other distinctive features (unusual colour, exudates, fluorescence, a transparent and cracked layer on the surface of the mantle).

Pieces of mantle that were scraped off ectomycorrhizas were far easier to study and interpret than whole squash mounts. Depth of field could be increased to show internal details without the interference of host tissues, and the inner surface of the mantle was examined directly.

Scraping, which results in many small pieces of mantle, may even have some advantage over peeling off a few large sections. Mantle layers, cystidia or emanating hyphae are often seen in isolation at the edge of mantle pieces and thus are more easily examined.

Besides providing additional characters, the anatomy of an ectomycorrhiza can be related to its morphology. Understanding morphological appearance in terms of anatomical structure can help one determine which morphological features are significant and likely to be consistent. The six classes of mantle anatomy of Ingleby *et al.* (1990) were an excellent framework. The mantle surface of some types spanned three of these classes, although a predominant class could be chosen. Variation within a tip could be due to age, although tissue within 2 mm of the apices of roots was not examined. Agerer (1993) made a significant advance by describing 16 types of mantle anatomy that include variations within Ingleby's classes, combinations of them, and the presence of surface features. I recommend that both systems be used in a hierarchical manner, *ie.*, that a mantle be designated according to Ingleby's system and, if possible, more precisely according to the classes of Agerer. Further study of ectomycorrhizas will identify more mantle types.

Loosely speaking, a mantle or an ectomycorrhiza may be called prosenchymatous or synenchymatous according to the anatomy of its outer surface, because there is little variation among the inner surfaces of ectomycorrhizas, and any tissue between is almost always like either the outermost or innermost cell-layers. Technically, however, the classes of mantle anatomy refer only to a specified layer within the mantle. It is not surprising that the innermost layer of the mantle is mostly a net synenchyma, as it can also be considered the outermost part of the Hartig net.

For some types the thickness of the mantle is consistently and obviously very thick or very thin. It might be useful to quantify this. Cross-sections of

ectomycorrhizas could also be used to examine features of the Hartig net, the cortical cells and the tannin-layer. An accurate picture of rhizomorph anatomy also requires cross-sectioning.

Figure 1.14 supports somewhat the idea of a continuum of morphologies of ectomycorrhizas ranging from prosenchymatous with lack of organization in mantles and mycelial strands to synenchymatous with thin mantles of modified cells and rare or absent strands (a morphostructural series) (Godbout and Fortin 1985a). This generalization may simply aid in recognition and description of ectomycorrhizas, or it may provide clues to how different types may be functionally distinct. Why are rhizomorphs rare or absent on synenchymatous ectomycorrhizas? Is this simply a combination of evolutionary advanced traits? And why do many prosenchymatous mantles also lack rhizomorphs? How do prosenchymatous and synenchymatous mantles function differently? Presumably, ectomycorrhizas with compact mantles are better able to control the passage of materials into and out of the mantle and Hartig net. It is interesting that round, compact rhizomorphs with an outer rind and central hyphae like vessels tend to be attached to compact mantles and are less abundant than simpler rhizomorphs. The degree of differentiation of rhizomorphs is inversely related to their abundance, perhaps because more complex rhizomorphs are more efficient transporters or because they access more restricted or distant substrates. It is still unclear whether some ectomycorrhizas are naturally without rhizomorphs and emanating hyphae, or if all ectomycorrhizas have at least some but these are inadvertently removed during sampling, washing or cleaning. I have carefully dissected intact soil masses of fragmented material and of decayed wood containing ectomycorrhizas (e.g. em6 and em14) without encountering rhizomorphs. My suspicion is that some fungi form ectomycorrhizas without rhizomorphs, or with very rare rhizomorphs. Many ectomycorrhizas have been extensively studied and never observed to form rhizomorphs (Danielson R.M. 1995, personal

communication). Perhaps these fungi use unobserved emanating hyphae to procure nutrients from the soil, or perhaps they have niches that do not require rhizomorphs.

The presence or absence of clamp connections was consistent in each type and was easily ascertained. Some types consistently had both clamped and non-clamped septa. When septa were visible in a net synenchyma they were clampless even if hyphae elsewhere were clamped. Thus to see if clamps are present on fungi that form synenchymas, emanating hyphae must be observed. It would be useful to know to what extent the type of ectomycorrhizas formed by monokaryons of a fungus differ from those formed by dikaryons. Types were not found that differed only in the presence or absence of clamps. The size and shape of clamps may be too variable in most types to be easily used. The use of this character is limited because observation of septa is difficult for those types with few or no emanating hyphae and a synenchymatous mantle. The proportion of ectomycorrhizas on which clamps were observed is for this reason not necessarily indicative of the proportion of clamped fungi within the mycosymbionts.

Hyphal ornamentation can be distinctive and is usually characteristic of a type, but presents some problems. Ornamentation may not always be present, perhaps due to the age of the ectomycorrhizas or their environment. Ornamentation may be found in rhizomorphs but not the mantle, or vice versa. Older hyphae can appear very different than younger hyphae as a result of accumulation of surface deposits. Younger hyphae may be smooth.

Setae and cystidia were key features if distinctive and in abundance. In some types infrequent cystidia-like hyphal branches were observed that were short, variable and not as consistently observed. These were difficult to interpret.

The substrate in which ectomycorrhizas is found (e.g. mineral soil, wood, litter, fragmented needles) could be a useful character. Some types were found more often in some substrates than others (see chapter 3). Features of mycosymbionts in culture, although not features of

ectomycorrhizas, could help identify the fungus.

The most important characters used to develop a key to types were similar to those used by others (Agerer 1993, Jansen and de Vries 1988, Roth 1990): colour, presence of clamps, type of outer mantle, rhizomorph presence and type and laticiferous hyphae. Agerer (1993) used more anatomical details requiring very careful work with the compound microscope. Unlike Agerer, Jansen and de Vries (1988) and I used hyphal ornamentation and surface texture, which are more quickly ascertained. Roth (1990) used several features in his key not used in other keys, including mantle thickness, distance between septa and details of the Hartig net. Because of these differences, comparison of the descriptions made in this study with those made previously is not facilitated by the keys. The key presented in Appendix B was developed to help distinguish the types I found and is most applicable to the forest ecosystem of this study. Despite problems describing colour, it is a good starting point because like other morphological features, it is quickly discerned for a large number of tips. When using the key it may be useful to run brown and white types through both the white and the brown sections of the key. The key could be improved by placing some types that are both brown and white in both sections. Anatomical characters are in some cases less variable, but could not practically be ascertained for more than a few tips due to the time required to prepare and examine slide mounts. This is a consideration for those anatomical characters such as mantle type and hyphal ornamentation that are more variable than others and may be no more reliable than colour.

The database of type descriptions could be converted to a form accessible to computer programs such as DELTA (description language for taxonomy) (Askevold and O'Brien 1994), which can generate a variety of types of keys. This could be used to test alternative structures, such as using mantle type as the first level of division. Most of the types included in the key are based on only a few samples or a few tips. The key could be simplified by removing these

tentatively described types, leaving only well-defined, consistent and common types.

Linkage was useful as a method of identifying types. All sporocarps taken had tightly interwoven ectomycorrhizas in a dense mass of mycelium directly at the base of the mushroom. However, the ectomycorrhizas found with mushrooms did not quite match the types. This may be because only a few sporocarps were examined, because of variation within species, or because ectomycorrhizas formed by a fungus at the base of sporocarps are different from ectomycorrhizas formed elsewhere by the same individual. The latter case might occur as a result of a transfer of materials from ectomycorrhizas to the developing sporocarp, although R. Agerer (1995, personal communication) and R.M. Danielson (1995, personal communication) have found no evidence of this. Comparison of types with published descriptions will become more feasible as a way of identifying ectomycorrhizas as more descriptions are published.

This primary objective of this study was not to describe and classify types, but rather to compare the diversity of ectomycorrhizas in several habitats. Thus there was a strategic tradeoff between number of samples processed and the completeness of descriptions. To save time, fewer characters were measured than recommended for taxonomic purposes. Nevertheless, the descriptions of ectomycorrhizas of Douglas-fir produced here are sufficiently detailed to clearly distinguish 30-40 types, which, along with previous descriptions (Chu-Chou and Grace 1983a, Froidevaux 1975, Hunt 1991, Molina and Trappe 1982, Roth 1990, Trappe 1967, Zak 1969, 1971a, Zak and Larsen 1973), contribute to future ecological or taxonomic research.

**Tables and figures**

Table 1.1. Ectomycorrhizal taxa

Subphylum	Order or group	Family or genus	Mycorrhizal status
Basidiomycotina (Miller 1982, 1983; Danielson R.M. 1995, personal communication)	Aphyllorphorales	Cantherellaceae	all?
		Thelephoraceae	most
		Hydnaceae	
		Clavariaceae	
		Polyporaceae	few (e.g. <i>Byssoporia</i> (Zak and Larsen 1978))
	Agaricales	Amanitaceae	all?
		Hygrophoraceae	most
		Russulaceae	all?
		Tricholomataceae	
		Entolomataceae	
		Cortinariaceae	
		Paxillaceae	most
		Gomphidiaceae	all?
		Boletaceae	
		Strobilomycetaceae	
	Phallales		few (e.g. <i>Hysterangium</i> )
	Gasteromycetes	Melanogastraceae (e.g. <i>Alpova</i> , <i>Melanogaster</i> )	all?
		Leucogastraceae (e.g. <i>Leucogaster</i> , <i>Leucophleps</i> )	
		Astraceae (e.g. <i>Astraeus</i> )	
Sclerodermataceae (e.g. <i>Scleroderma</i> , <i>Pisolithus</i> )			
Gautieriaceae (e.g. <i>Gautieria</i> )			
Rhizopgonaceae (e.g. <i>Rhizopogon</i> )			
Ascomycotina (Trappe 1971)	<i>Elaphomyces</i>	all?	
	<i>Genea</i>		
	<i>Hydnotyra</i>		
	<i>Tuber</i>		
	<i>Helvella</i> ?		
	<i>Otidea</i> ?		
	<i>Geopora</i>		
<i>Gyromitra</i> ?			

**Table 1.2. Characters recorded for ectomycorrhizal types  
(adapted from Agerer 1993 and Ingleby et al. 1990)**

**General Morphology**

- type of ramification of ectomycorrhizal root tips (6X): unramified, monopodial-pinnate, monopodial-pyramidal, dichotomous\*, irregularly pinnate, coralloid, tuberculate
- length of system of ectomycorrhizal root tips (6X)
- diameter of tips (40X)
- diameter of axes (40X)
- shape of tips: straight, bent, tortuous, beaded\* (6X)
- distinct mantle surface visible? (y/n) (6X)
- cortex visible through mantle? (y/n) (6-40X)
- surface appearance or texture (6-40X): smooth shiny, smooth mat, reticulate, grainy, warty, woolly, cottony, felty, stringy, short spiny, long-spiny
- Colour of apex, tips and older parts in daylight quality light (singly or as combinations) (6X): white, brown, yellow, black, grey, blue, green, pink, orange, red

**Morphology of rhizomorphs**

- frequency (6X): abundant, common, occasional, none
- position of attachment (6X): apical, subapical, etc.
- connection with mantle (6X): restricted point of attachment, growing from larger area of attachment at angles < 45 degrees, hyphal fans
- colour (6X): as above
- diameter (40X)
- frequency and angle of ramification
- shape in x-section: flat, round, variable
- appearance: loosely constructed of interconnected filaments, fleshy (compact) with some stands of hyphae attached (hairy), fleshy (compact) and smooth

**Morphology of emanating hyphae**

- density: abundant, common, occasional, none
- occurrence: everywhere, at apex, etc.
- shape: wispy, straight, gently curved, woolly, felty, wobbly (tortuous), cottony, rat's nest (tangled), cob-webby, fuzzy

**Sclerotia**

- present? (y/n)

**Colour reactions of whole ectomycorrhizas to chemical reagents**

- 70% Ethanol
- 10% FeSO<sub>4</sub>

- 15% KOH
- 85% lactic acid
- Meltzer's reagent
- sulfo-vanillin

**Autofluorescence** (whole ectomycorrhizas, colour if present):

- @ 254nm
- @ 366nm

**Anatomy of mantle (1000X):**

- A. Laticiferous Hyphae.
  - distribution
  - diameter ( $\mu\text{m}$ )
  - ramification: angle, frequency
  - contents: granular, clear
  - colour
  - septa: frequency, etc.
- B. Outer Surface
  - tissue type: 1. felt prosenchyma (hyphae like those emanating, 2. net prosenchyma (hyphae wider, more frequently branched than those emanating, maybe joined like a net, 3. net synenchyma (elongated branched cells), 4. interlocking irregular synenchyma (epidermioid) (jigsaw), 5. non-interlocking (roundish cells), 6. regular synenchyma (polygonal cells)
  - mantle thickness: single cell layer, thin, thick
  - Cell features:
    - shape
    - length
    - width
    - thickness of walls
    - colour
    - surface: smooth or with ornamentation or deposits of various shapes sizes and densities
    - septa: frequency, shape
    - clamps: frequency, shape
- C. Inner Surface: as for the outer surface

**Anatomy of rhizomorphs (1000X)**

- differentiation of hyphae: presence of vessel-like hyphae, or specialized surface hyphae
- hyphal characters: arrangement, size, shape, ornamentation, branching, septa, anastomoses, contents, thickness of walls

**Emanating hyphae (1000X):**

- shape: straight, curved, slightly curved, wiggly, kinky, with elbows, bumpy, wispy, etc.
- septa
- clamps? (y/n)

- diameter ( $\mu\text{m}$ )
- wall thickness
- colour
- surface: smooth or with ornamentation or deposits or various shapes sizes and densities
- ramification: frequency, angle, shape of junction
- proximal ends: differences in size from other parts of the hyphae
- distal ends: shape of tips, differences in size, shape, surface and content
- frequency of anastomoses
- type of anastomoses

**Cystidia (1000X)**

- presence (y/n)
- shape
- length
- width ( $\mu\text{m}$ )
- wall thickness ( $\mu\text{m}$ )
- content
- colour
- surface

**Chlamydo spores (1000X)**

- shape, size

\* not observed.

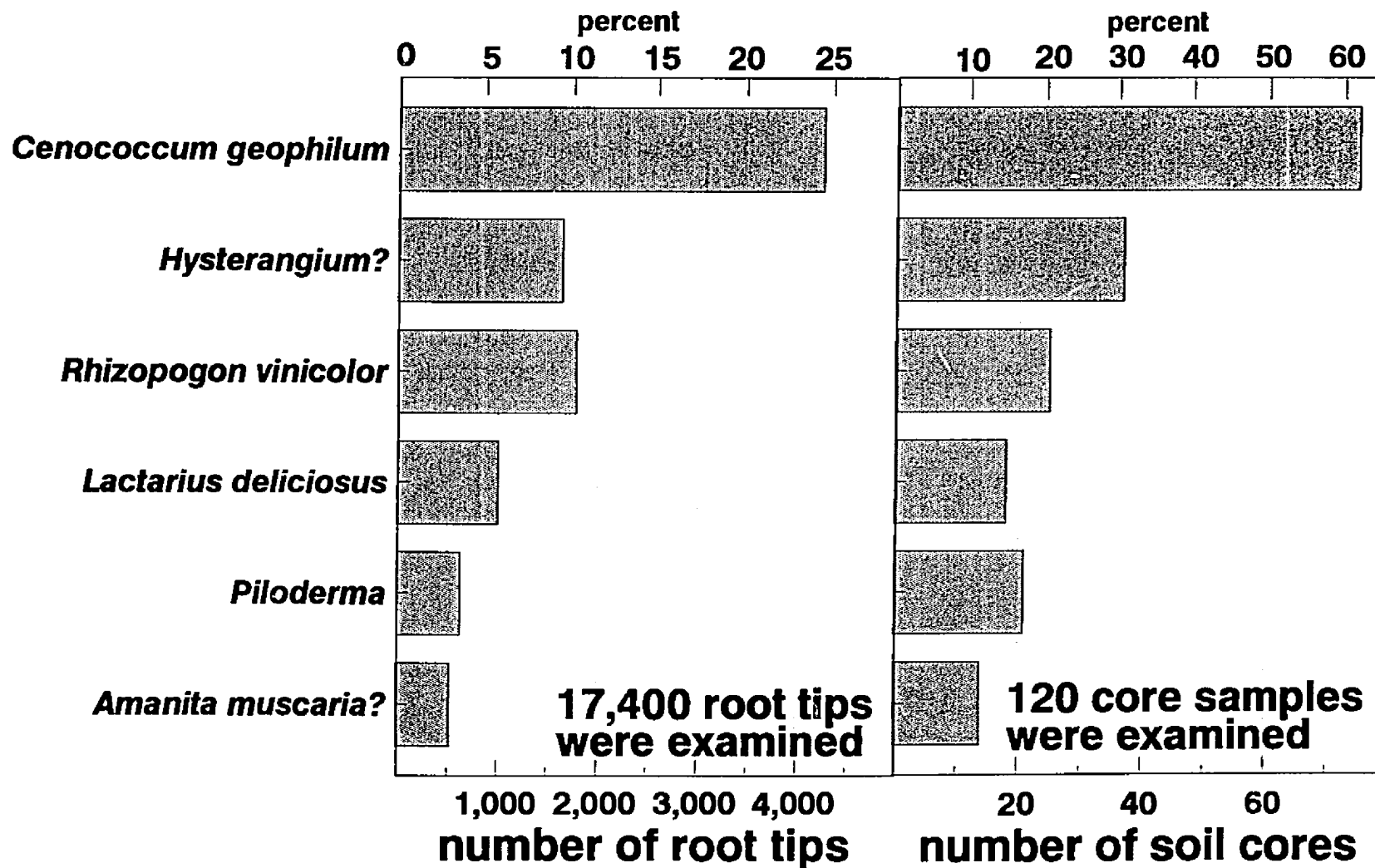


Figure 1.1. Abundance of co-dominant ectomycorrhizal fungi in terms of number of root tips colonized and number of samples of soil in which they occurred

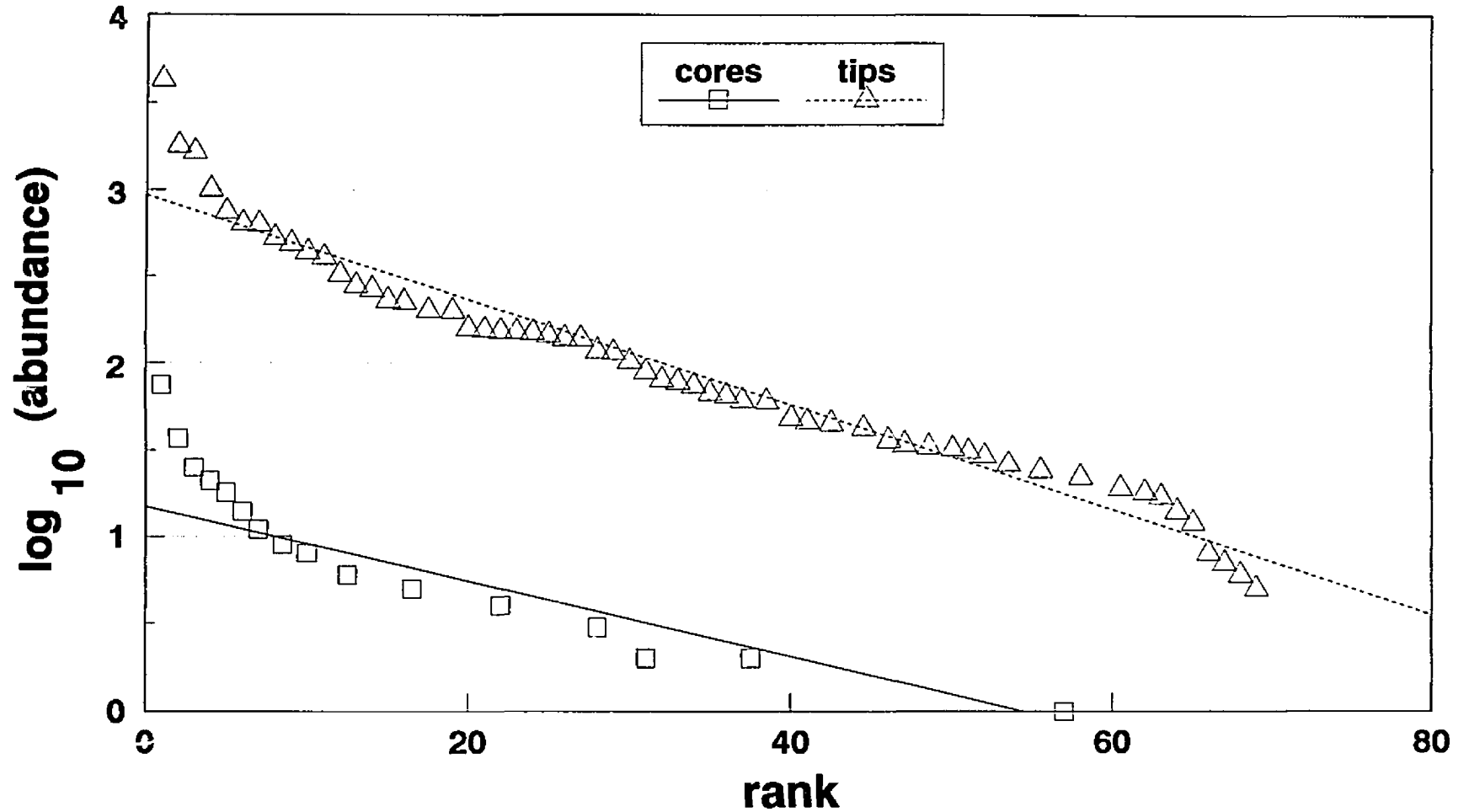
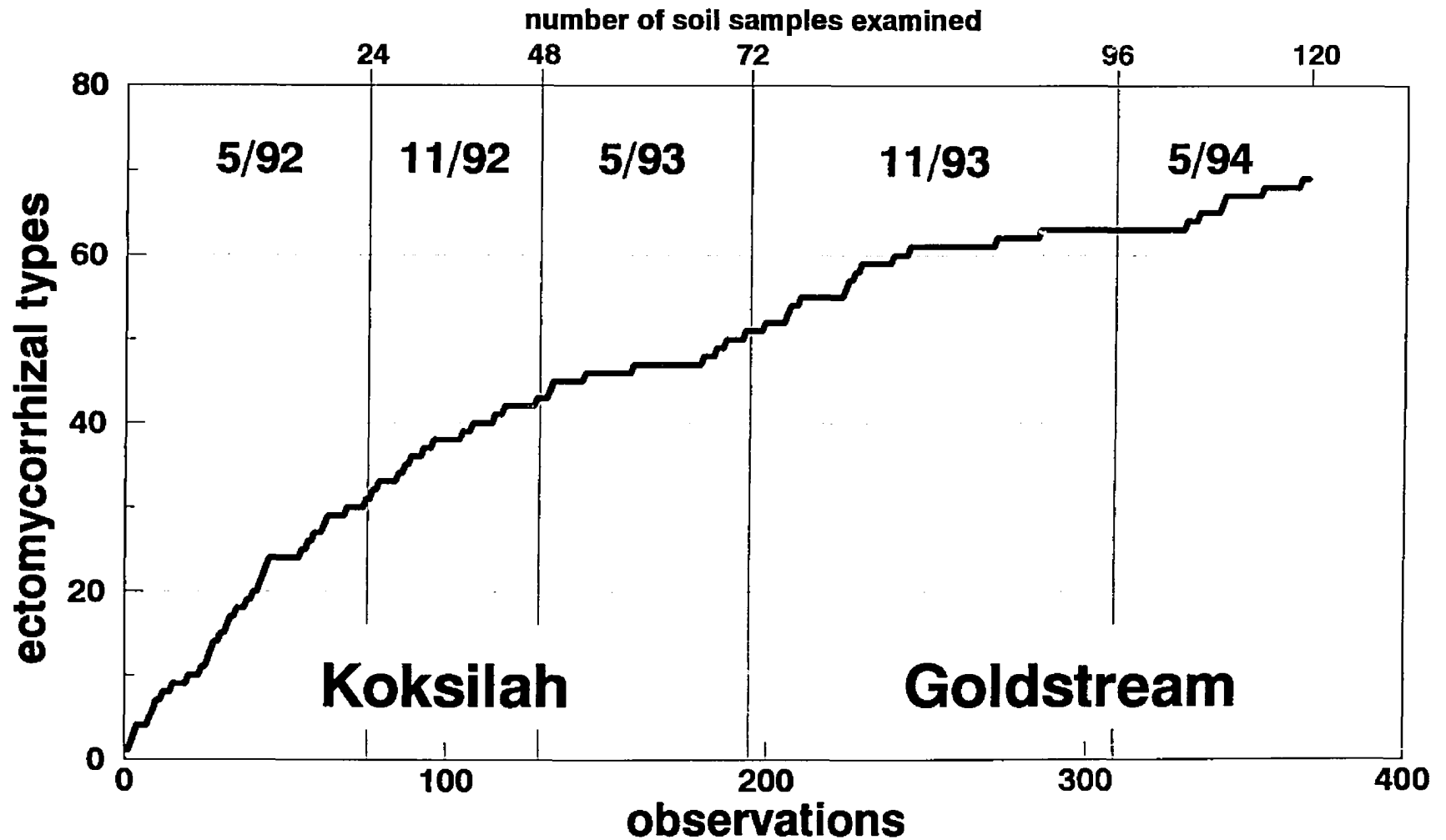


Figure 1.2. Equitability–abundance of ectomycorrhizas in terms of number of root tips colonized and number of core samples in which each type was observed as a function of rank by abundance of cores and tips. Some points represent a group of types with a tied rank.



**Figure 1.3. Number of ectomycorrhizal types distinguished as a function of the cumulative number of observations of types (an observation is the occurrence of a type in a core sample). Dates of sampling are indicated above.**

**Figure 1.4. Morphological types of ectomycorrhizas for which colour is a distinguishing feature--types 3, 5, 6 and 7**

a) bright yellow tip and rhizomorphs of *Piloderma fallax* (type 3), scale bar = 1000  $\mu\text{m}$

b) tip of type 5 with a white grainy mantle and blue bruising, scale bar = 1000  $\mu\text{m}$

c) smooth, shiny, light-yellow tip of type 6, scale bar = 100  $\mu\text{m}$

d) part of tubercle of *Rhizopogon vinicolor* with the dark peridium partially removed, revealing a dark brown mycelium lining the inner surface of the peridium and white-mantled root tips, scale bar = 1000  $\mu\text{m}$



**Figure 1.5. Morphological types of ectomycorrhizas for which colour is a distinguishing feature--types 13 (*Lactarius deliciosus*), 14 and 20**

- a) smooth, shiny, light-brown tips of type 13, *Lactarius deliciosus* with bright green rhizomorph and green patches, scale bar = 1000  $\mu\text{m}$
- b) mantle of type 13, *Lactarius deliciosus*, with large and small orangish-yellow laticiferous hyphae in a net prosenchyma, scale bar = 100  $\mu\text{m}$
- c) light-brown, grainy tips of type 14, with dark surface bumps, scale bar = 1000  $\mu\text{m}$
- d) *Amphinema byssoides*, type 20, with brownish-yellow mantle and rhizomorphs, scale bar = 1000  $\mu\text{m}$



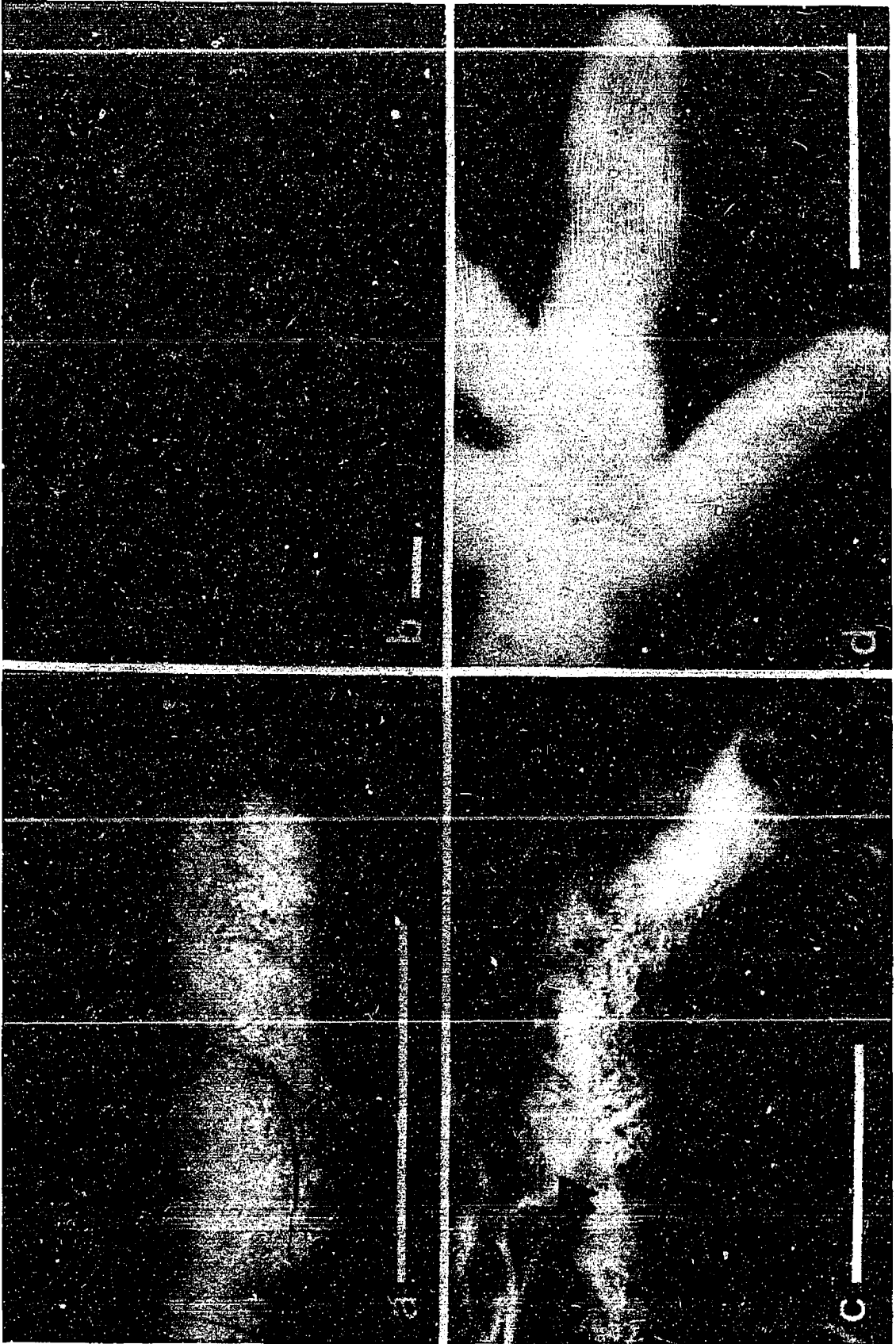
**Figure 1.6. Morphological types of ectomycorrhizas for which colour is a distinguishing feature--types 29, 46 and 65**

a) bright yellow, grainy to felty, tip of type 29, scale bar = 1000  $\mu\text{m}$

b) outer mantle of type 29, a net prosenchyma with greenish-yellow hyphal contents, scale bar = 10  $\mu\text{m}$

c) tip of type 46, with abundant rhizomorphs and a reticulate prosenchymatous mantle with some brownish pink colour (arrow), scale bar = 1000  $\mu\text{m}$

d) finely woolly to reticulate, light-brown tips of type 65, scale bar = 1000  $\mu\text{m}$



**Figure 1.7. Distinguishing features of common or distinctive morphological types of ectomycorrhizas-type 1 (*Cenococcum geophilum*) and type 2 (*Hysterangium*)**

a) net synenchymatous outer mantle of *Cenococcum geophilum*, showing a stellate (radiating) pattern of dark-brown cells with central setae, scale bar = 100  $\mu\text{m}$

b) scanning electron micrograph of *Cenococcum geophilum*, showing a stellate (radiating) pattern of cells with central setae

c) inner surface of the mantle of *Cenococcum geophilum*, with a net synenchyma of narrow dark-brown cells, scale bar = 10  $\mu\text{m}$

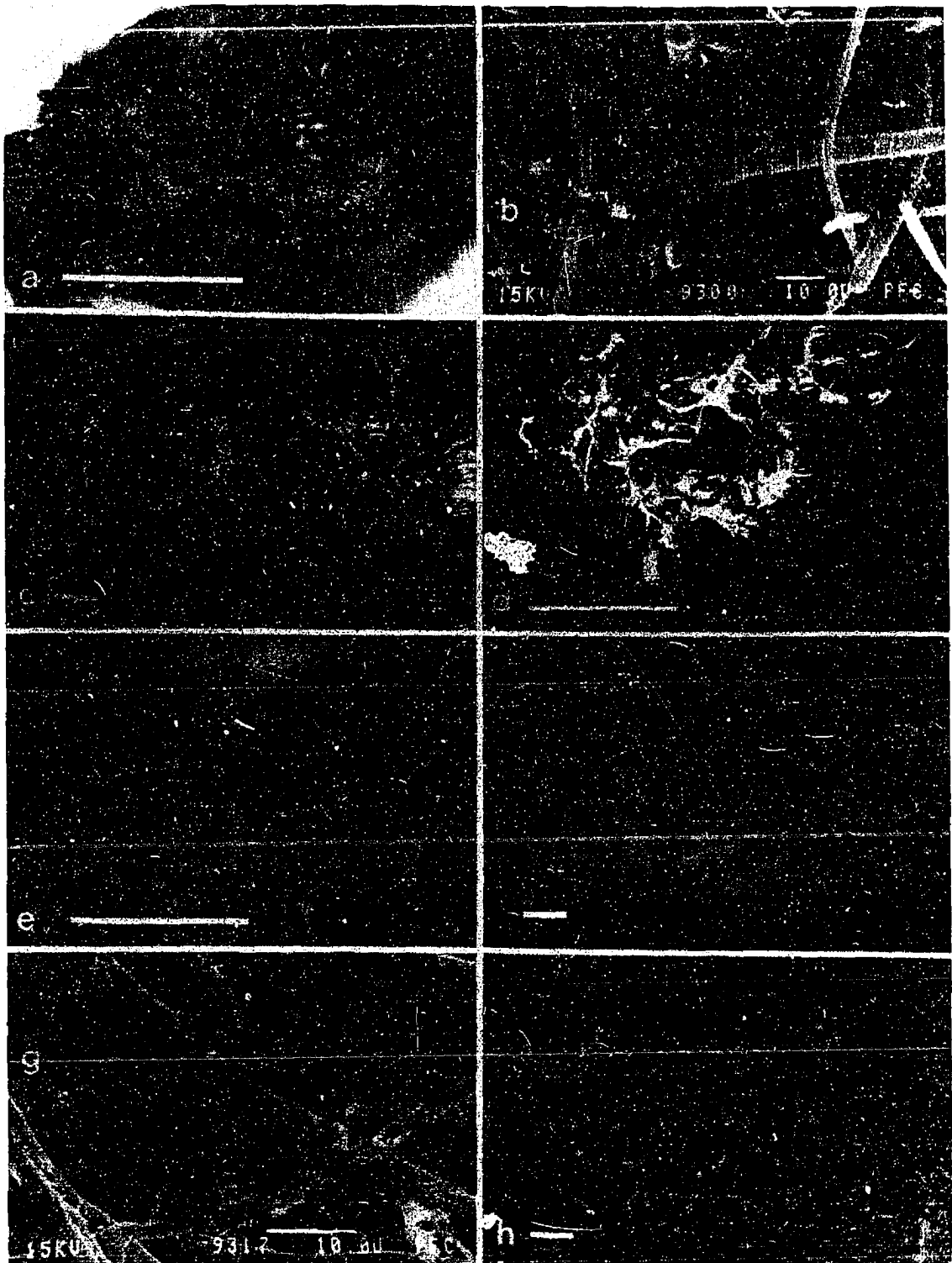
d) system of root tips of *Hysterangium*, prosenchymatous with abundant rhizomorphs, scale bar = 10000  $\mu\text{m}$

e) outer mantle of *Hysterangium*, a net prosenchyma of hyphae heavily encrusted, scale bar = 100  $\mu\text{m}$

f) emanating hyphae of *Hysterangium*, ornamented with elongated crystals, and with an H-shaped anastomoses, scale bar = 10  $\mu\text{m}$

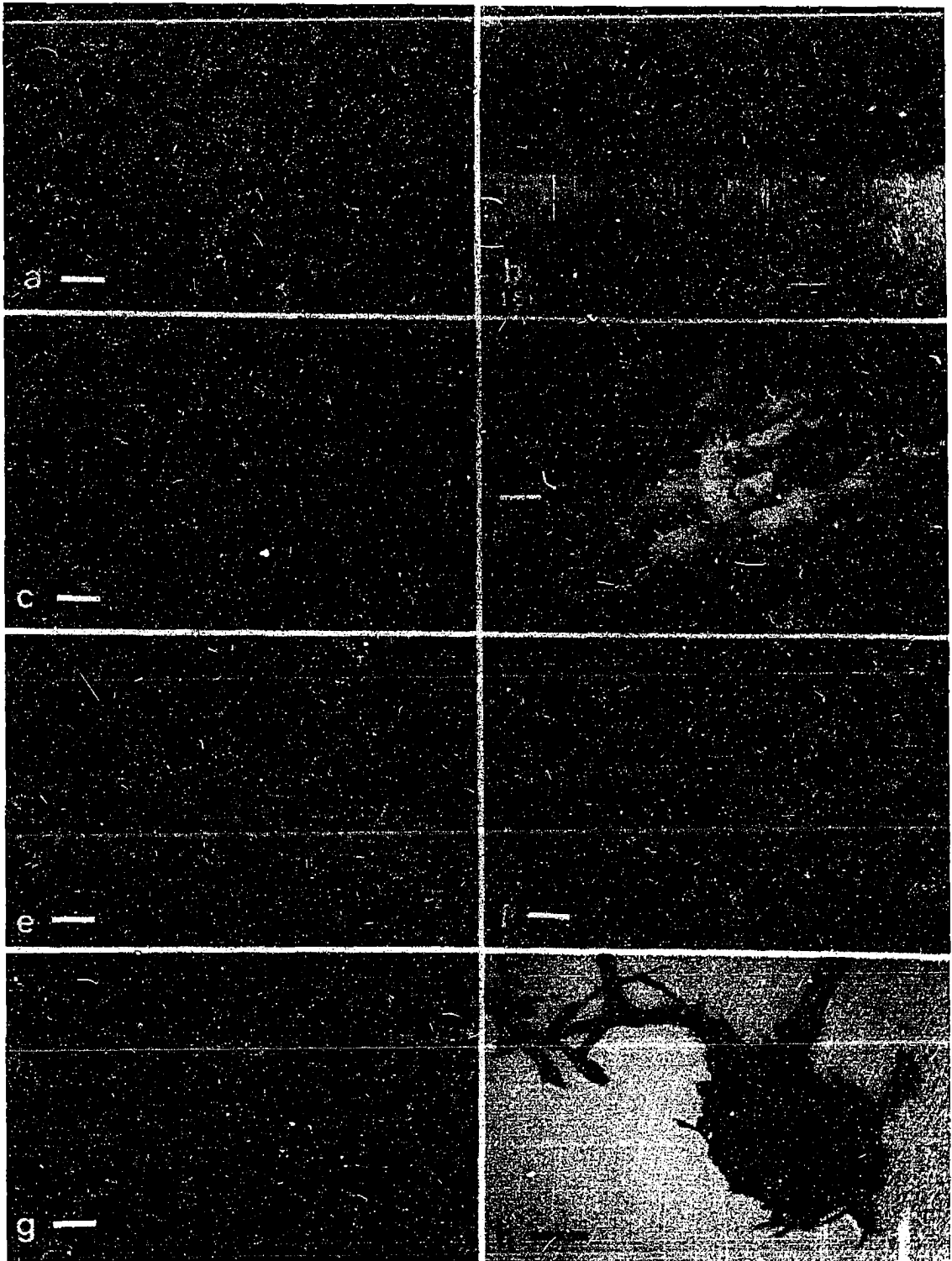
g) scanning electron micrograph of the outer mantle of *Hysterangium*, with narrow hyphae ornamented with elongated crystals

h) smooth hyphae of loosely-organized rhizomorph of *Hysterangium*, note the H-shaped anastomoses (arrow)



**Figure 1.8. Distinguishing features of common or distinctive morphological types of ectomycorrhizas-types 3 (*Piloderma fallax*), 5, 6 and 7 (*Rhizopogon vinicolor*)**

- a) straight to gently curved, yellow emanating hyphae of *Piloderma fallax*, with fine roundish ornamentation and an H-shaped anastomoses, scale bar = 10  $\mu\text{m}$
- b) Scanning electron micrograph of hyphae of the outer mantle of *Piloderma fallax*, with fine, roundish ornamentation
- c) prosenchymatous inner mantle of *Piloderma fallax*, with groups of parallel hyphae, scale bar = 10  $\mu\text{m}$
- d) system of type 5, with common, cylindrical rhizomorphs, and regularly pinnate branching pattern, scale bar = 1000  $\mu\text{m}$
- e) prosenchymatous outer mantle of type 5, with many short branches of narrow hyphae, scale bar = 10  $\mu\text{m}$
- f) prosenchymatous outer mantle of type 6, with barely visible, hyaline hyphae in an apparent gel-like matrix, scale bar = 10  $\mu\text{m}$
- g) net synenchyma of type 6, with hyphae and numerous small oil droplets or granular bodies in an apparent gel-like matrix, scale bar = 10  $\mu\text{m}$
- h) tubercle of *Rhizopogon vinicolor*, type 7, with a secondarily thickened root attached (top-right) and a ramified rhizomorph attached (top-left), scale bar = 1000  $\mu\text{m}$



**Figure 1.9. Distinguishing features of common or distinctive morphological types of ectomycorrhizas-types 7 (*Rhizopogon vinicolor*), 8 and 9**

a) narrow dark-brown hyphae of the felty mycelium lining the inside of the peridium of *Rhizopogon vinicolor*, note the "elbows" (e.g. arrow), scale bar = 10  $\mu\text{m}$

b) outer mantle of *Rhizopogon vinicolor*, a net prosenchyma, scale bar = 10  $\mu\text{m}$

c) tips of type 8, with a thick reticulate prosenchymatous mantle with abundant emanating hyphae, scale bar = 1000  $\mu\text{m}$

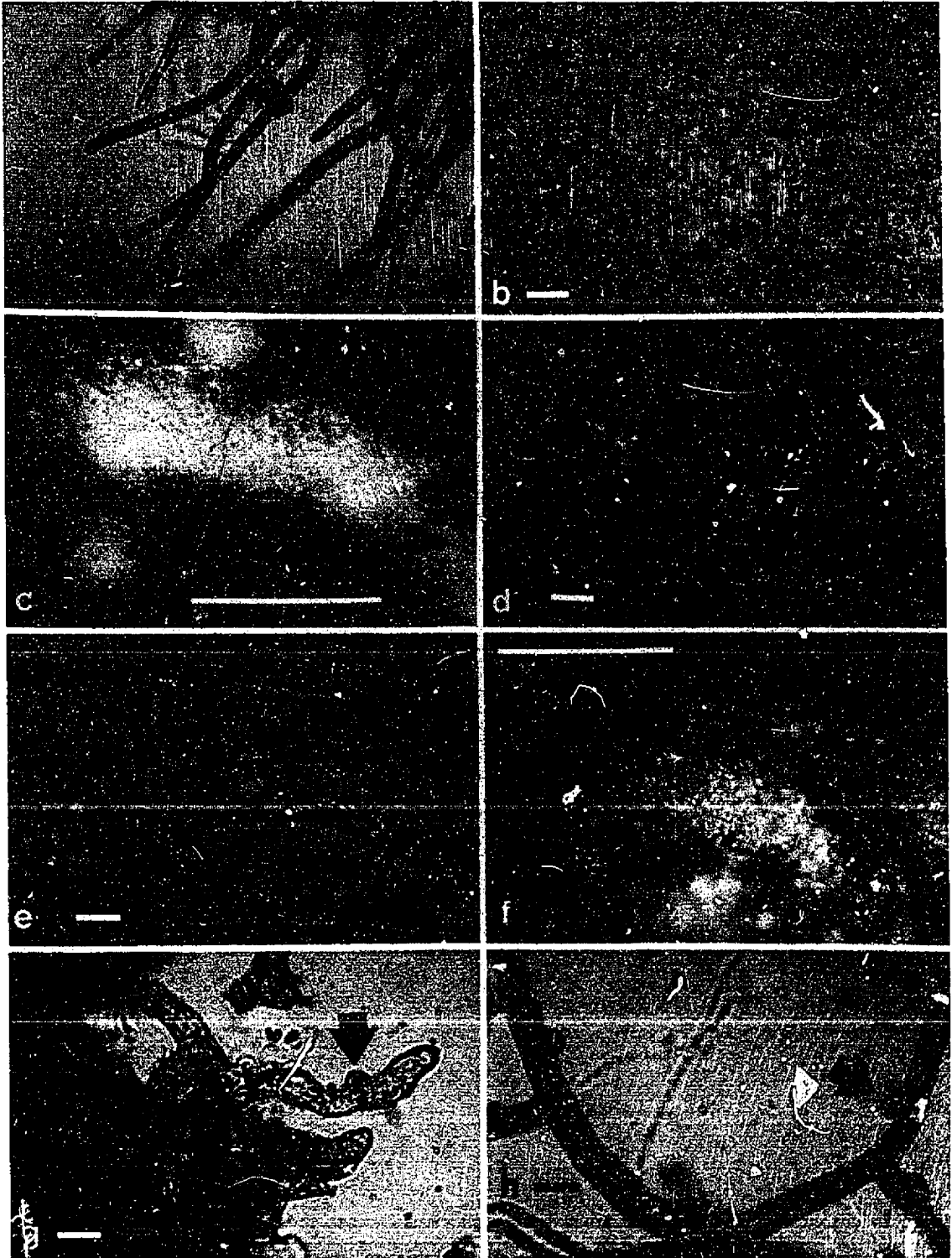
d) prosenchymatous outer mantle of type 8, with wide, smooth hyphae, scale bar = 10  $\mu\text{m}$

e) wide, smooth, clamped (e.g. arrow) emanating hyphae of type 8, scale bar = 10  $\mu\text{m}$

f) grainy to finely felty, dark brown tips of type 9, scale bar = 1000  $\mu\text{m}$

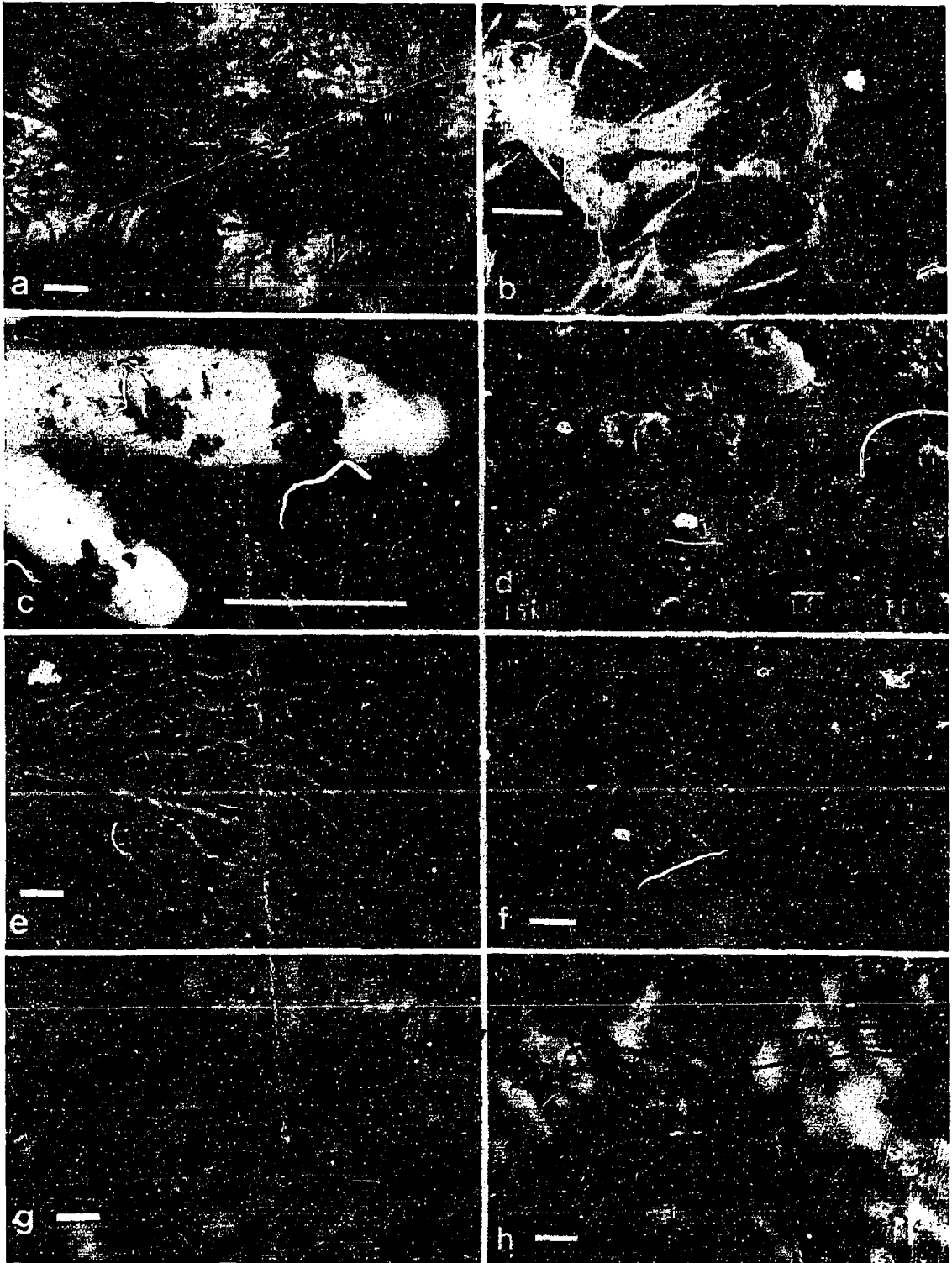
g) wide, clamped (e.g. arrow), brown, ornamented hyphae of the net prosenchyma of the outer mantle of type 9, scale bar = 10  $\mu\text{m}$

h) wide, clamped (e.g. arrow), brown, smooth to finely ornamented emanating hyphae of type 9, scale bar = 10  $\mu\text{m}$



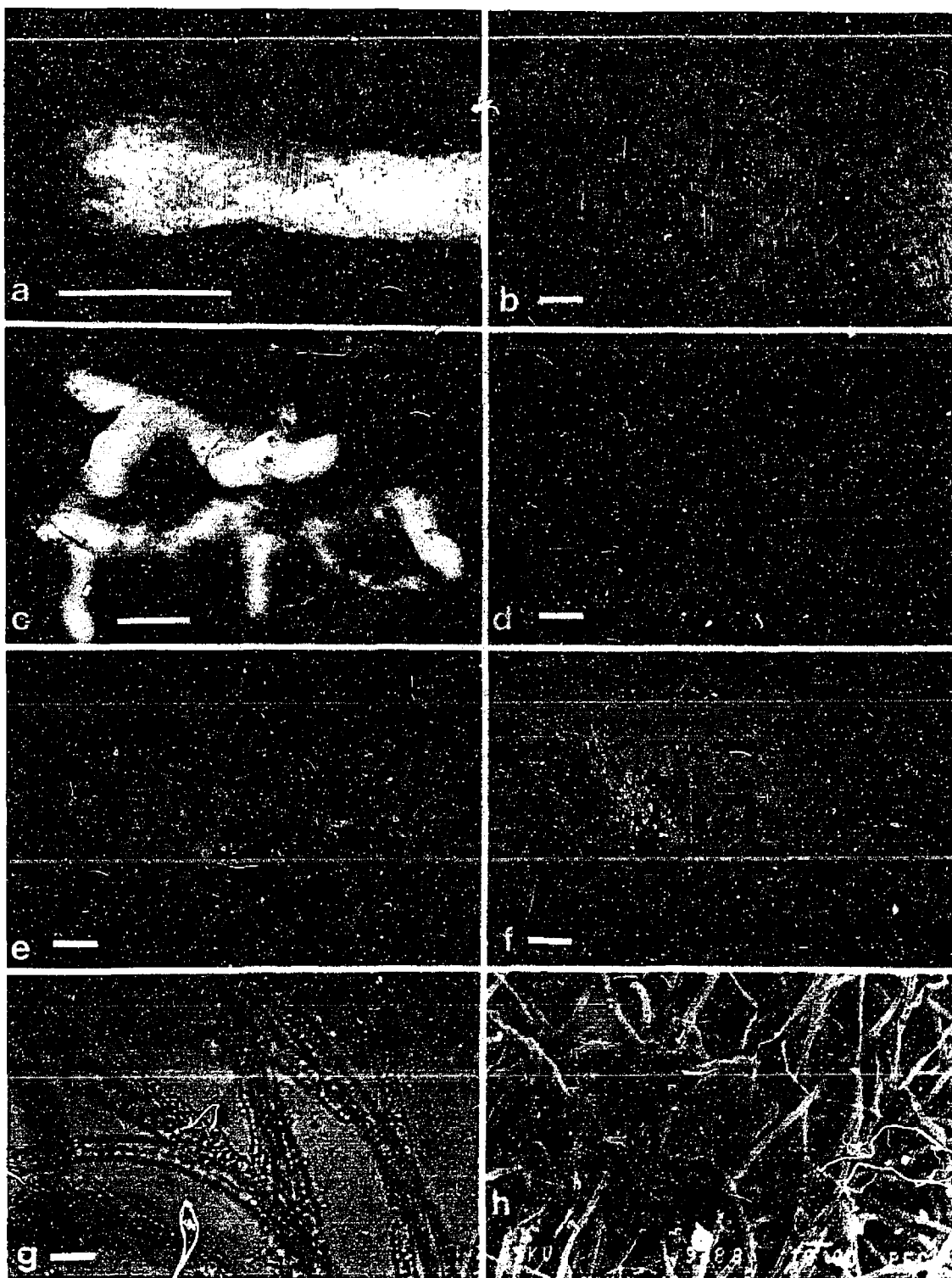
**Figure 1.10. Distinguishing features of common or distinctive morphological types of ectomycorrhizas--types 10, 12 and 14**

- a) a net synenchyma of radiating brown cells in the inner mantle of type 9, scale bar = 10  $\mu\text{m}$
- b) system of whitish prosenchymatous tips of type 10 with abundant loosely organized rhizomorphs, scale bar = 1000  $\mu\text{m}$
- c) light-brown tips with small white "warts", type 12, (brown material adhering to tips are organic soil particles), scale bar = 1000  $\mu\text{m}$
- d) scanning electron micrograph of a "wart" of type 12, seen to be composed of fungal cells
- e) wiggly emanating hyphae of type 12, scale bar = 10  $\mu\text{m}$
- f) wall papillae inside a cortical cell attached to the inner mantle of type 12, scale bar = 10  $\mu\text{m}$
- g) outer mantle of type 14, with brown and black colour and a triangular seta (arrow), scale bar = 10  $\mu\text{m}$
- h) wide, thick-walled emanating hypha of type 14, over the outer mantle showing brown and black colour, scale bar = 10  $\mu\text{m}$



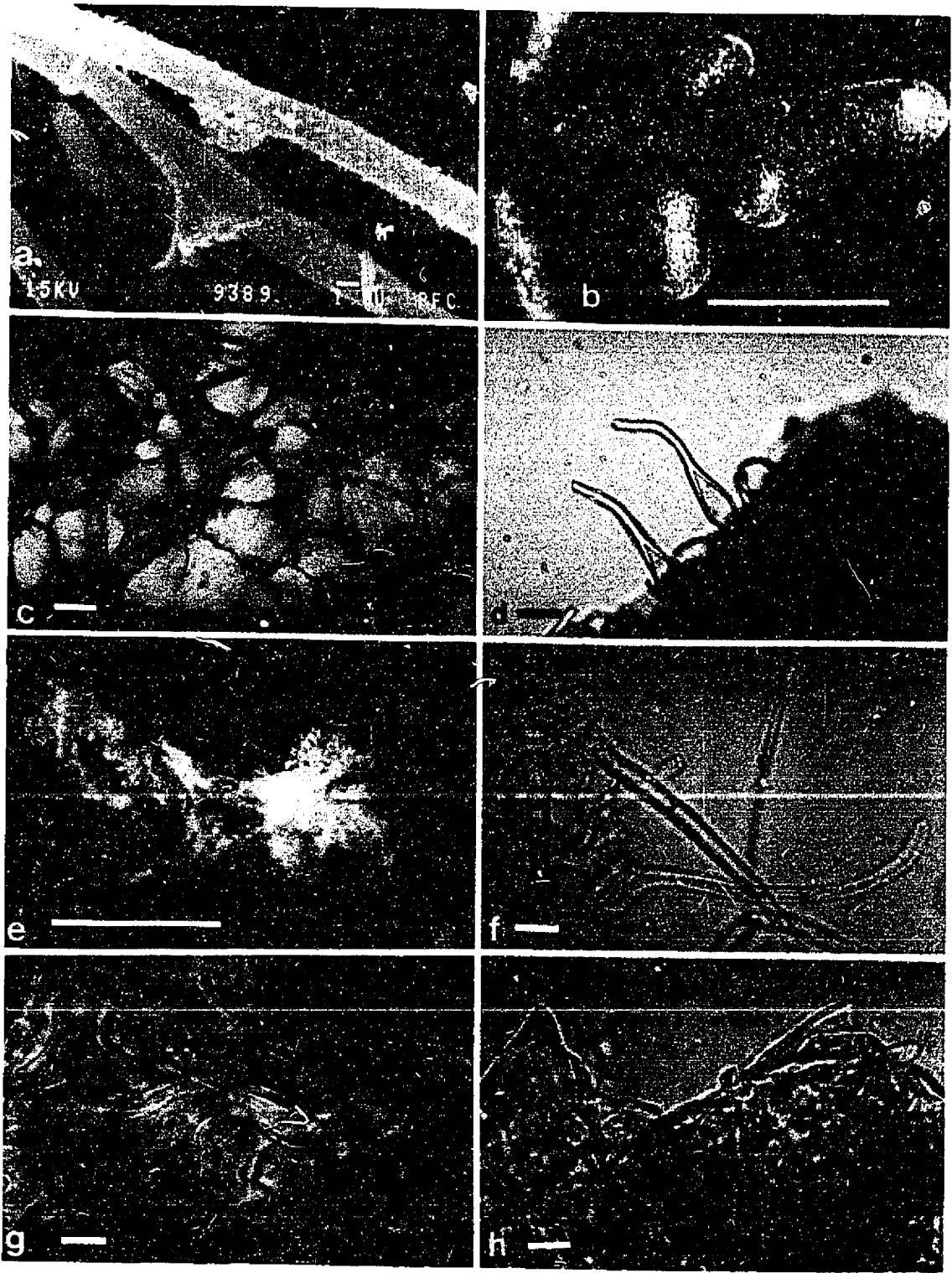
**Figure 1.11. Distinguishing features of common or distinctive morphological types of ectomycorrhizas--types 19, 21, 25, 27**

- a) white grainy tip of type 19, brown where surface hyphae have been rubbed flat, scale bar = 1000  $\mu\text{m}$
- b) outer mantle of type 19, a non-interlocking irregular synenchyma (roundish cells) that is somewhat interlocking, scale bar = 10  $\mu\text{m}$
- c) system of tortuous, smooth matte tips of type 21, scale bar = 1000  $\mu\text{m}$
- d) outer mantle of type 21, a regular synenchyma (polygonal cells), scale bar = 10  $\mu\text{m}$
- e) a net prosenchyma of clampless hyphae of type 22, scale bar = 10  $\mu\text{m}$
- f) outer mantle of type 25, an interlocking irregular synenchyma, scale bar = 10  $\mu\text{m}$
- g) emanating hyphae of type 27, heavily ornamented with roundish bodies, scale bar = 10  $\mu\text{m}$
- h) scanning electron micrograph of outer mantle of type 27, a felt prosenchyma



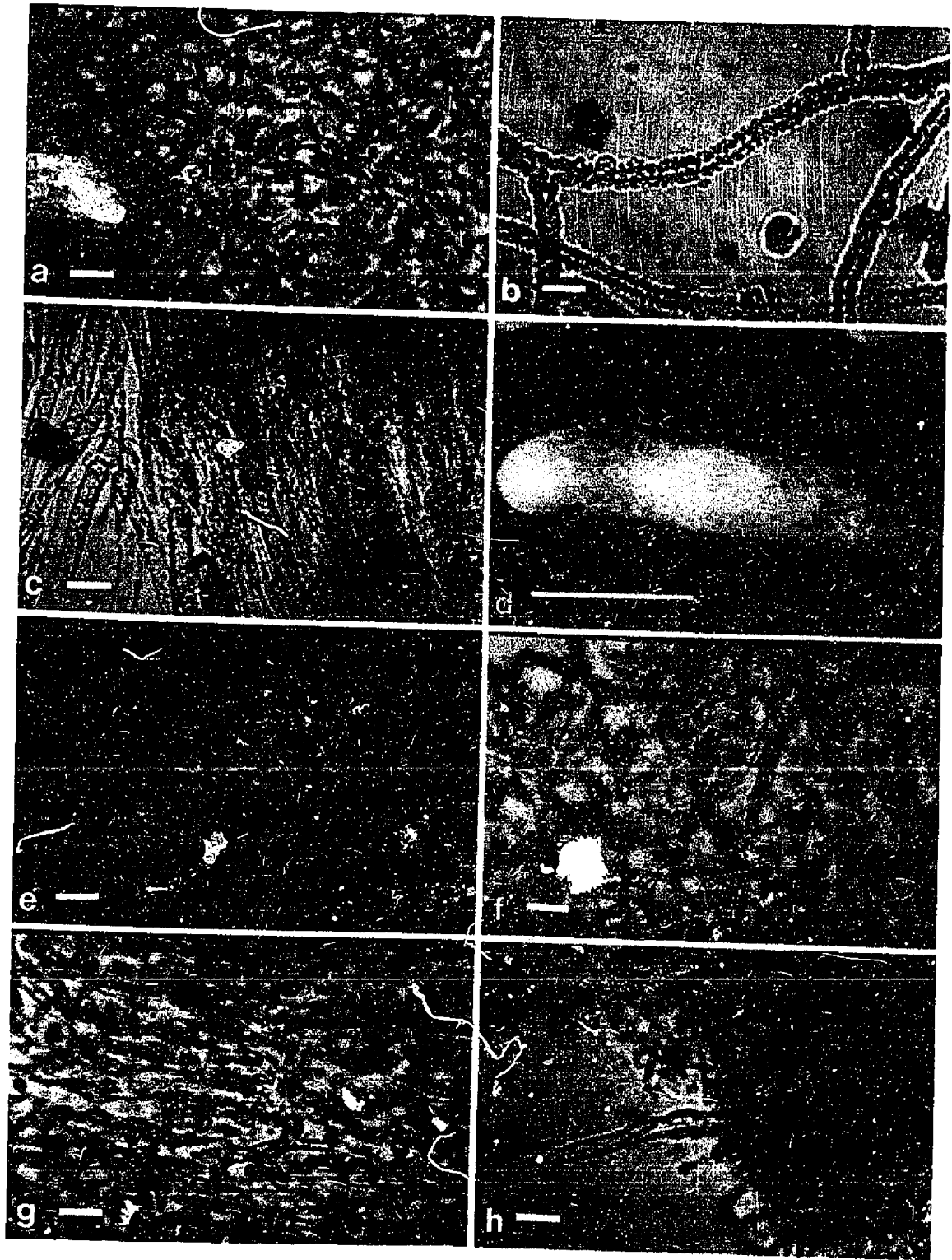
**Figure 1.12. Distinguishing features of common or distinctive morphological types of ectomycorrhizas--types 27, 28, 31, 42**

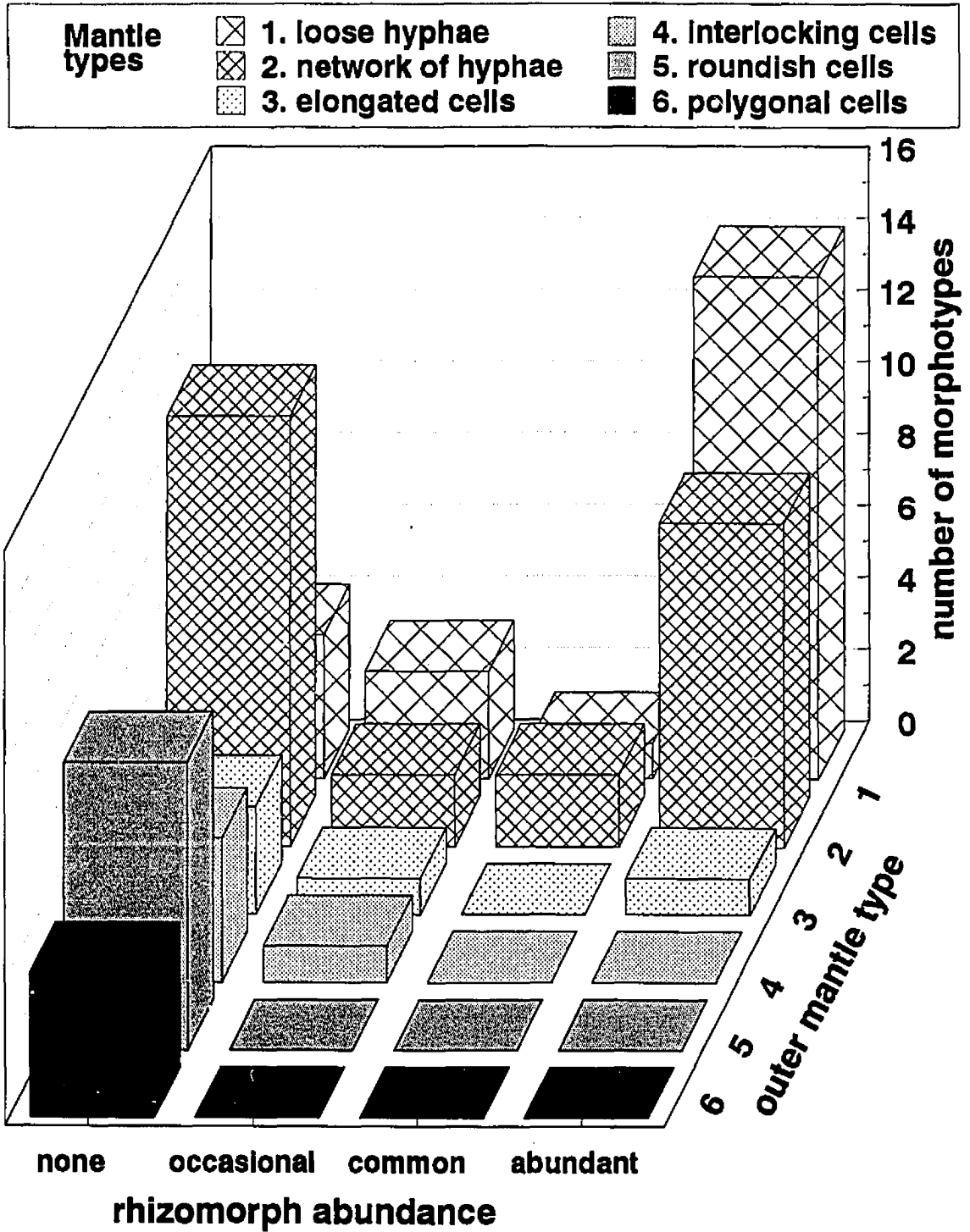
- a) scanning electron micrograph of clamped, finely ornamented hypha of type 27 in the outer mantle
- b) system of spiny dark brown tips of type 28, scale bar = 1000  $\mu\text{m}$
- c) a regular synenchyma, the outer mantle of type 28, scale bar = 10  $\mu\text{m}$
- d) cystidia of type 28, scale bar = 10  $\mu\text{m}$
- e) type 31, with white colour where cystidia have trapped air, scale bar = 1000  $\mu\text{m}$
- f) several septate, curved cystidia of type 31, with bases like other mantle cells
- g) a non-interlocking irregular synenchyma (roundish cells) of large thick walled cells, outer mantle of type 42, scale bar = 10  $\mu\text{m}$
- h) triangular, thick-walled cystidia of type 42, scale bar = 10  $\mu\text{m}$



**Figure 1.13. Distinguishing features of common or distinctive morphological types of ectomycorrhizas--types 43, 46, 57, 59, 62**

- a) outer mantle of type 43, a net synenchyma, scale bar = 10  $\mu\text{m}$
- b) emanating hyphae of type 43, clamped (arrows) and heavily ornamented with globules, scale bar = 10  $\mu\text{m}$
- c) rhizomorphs of type 46, with clamps (e.g. arrow) and brownish-pink granular ornamentation, scale bar = 10  $\mu\text{m}$
- d) yellowish-brown tip of type 57, with white laticiferous hyphae (many thin lines on mantle), scale bar = 1000  $\mu\text{m}$
- e) interlocking irregular synenchyma (epidermioid) of narrow, thick-walled cells, the outer mantle of type 57, scale bar = 10  $\mu\text{m}$
- f) surface of rhizomorph of type 59, with peculiar clear plates, scale bar = 10  $\mu\text{m}$
- g) surface of rhizomorph of type 59, with narrow, highly branched, purplish, hyphae, scale bar = 10  $\mu\text{m}$
- h) cystidium of type 62, *Russula aeruginea*, with a distinctive spore-like tip, in this case with two spore-like structures.





**Figure 1.14. Relationship between the structure of the outer layer of the mantle and abundance of rhizomorphs**

## Chapter Two -- Diversity of ectomycorrhizas in old-growth and mature stands

### Introduction

Of the once extensive old-growth forests of Douglas-fir on southeastern Vancouver Island only small fragments remain. The remaining stands continue to be cut, with unmeasured losses of biological diversity. Ectomycorrhizal fungi are of interest as they play an essential role in the growth of stands of Douglas-fir of all ages, and their sporocarps are valued as a recreational, aesthetic and commercial resource. Hence the need to assess the importance of old-growth stands of Douglas-fir for maintenance of diversity of ectomycorrhizal fungi.

Diversity and community composition of ectomycorrhizal fungi change with aging of (even-aged) forest stands. Several observers have noted a succession of ectomycorrhizas fruiting in young stands (<40 yr) (Gardner and Malajczuk 1985, Marks and Foster 1967, Mason et al. 1982, Visser and Danielson 1990). At least one study has shown that some succession of ectomycorrhizas might continue through later phases of stand aging--young (69 and 79 yr-old) and mature (84 and 130 yr-old) stands of Douglas-fir in Oregon differed in composition of hypogeously fruiting ectomycorrhizal species (Luoma et al. 1991). Reasons for ectomycorrhizal succession have not been established, although organic layer accumulation (Gardner and Malajczuk 1985, Visser and Danielson 1990) and changes in nitrogen availability (Abuzinadah et al. 1986, Finlay and Frostegard 1990) may be involved. As a forest stand ages there is a depletion of nitrogen in mineral soil and an accumulation of nitrogen in the organic layer (= forest floor), accompanied by a redistribution of fine roots (and ectomycorrhizas) to the

organic layer (Gessel et al. 1973, Grier et al. 1981).

The primary objective of this study was to compare the diversity of ectomycorrhizas in old-growth and mature stands of Douglas-fir.

## Literature Review -- Succession of ectomycorrhizas as forests age

1. **Methods of studying ectomycorrhizal succession.** Most surveys of fungal sporocarps and ectomycorrhizas in the same area have found many fungi fruiting but not forming ectomycorrhizas, or vice-versa (e.g. Chu-Chou 1979, Dahlberg and Stenstrom 1991, Danielson 1984, Lamb 1979, Visser and Danielson 1990). Fungi found fruiting abundantly but not forming mycorrhizas may have been saprotrophic, facultatively saprotrophic, or may have formed ectomycorrhizas that were not detected because they were too far from sporocarps. Failure to find epigeous sporocarps of common ectomycorrhizal fungi may have been because they were not in their fruiting season or year, because they were not physiologically or genetically capable of producing sporocarps, or because they were fungi that fruit hypogeously (truffle-formers). Observation of sporocarps provides limited information about succession of ectomycorrhizas.

Biologically assaying soil for ectomycorrhizas by planting trap-seedlings in a greenhouse is also, by itself, of little value (Borchers and Perry 1990, Deacon et al. 1983, Parke et al. 1984, Visser and Danielson 1990). In a greenhouse assay a similar set of ectomycorrhizas usually forms, even in soils known to contain different ectomycorrhizas. However, if trap seedlings are planted in the forest with minimal disturbance of the soil, they tend to form mycorrhizas with the dominant ectomycorrhizal fungi (Fleming 1983, 1984).

Surveys of naturally occurring ectomycorrhizas, combined with identification of the ectomycorrhizal fungi by comparing isolates from mycorrhizas and sporocarps, have provided the best evidence of ectomycorrhizal succession (Chu-Chou and Grace 1990, Deacon et al. 1983, Danielson 1984).

**2. Persistence of ectomycorrhizas on nursery seedlings after outplanting.** Compared to studies of older trees, studies of the succession of ectomycorrhizas on seedlings in the first year or two after planting involve fewer, more easily recognized types of ectomycorrhizas, requiring less detailed examination of roots (e.g. Dahlberg and Stenstrom 1991). Most fungi that form ectomycorrhizas in nurseries usually do not persist on the root systems of seedlings more than two years after outplanting (e.g. Benecke and Gobl 1974, Bledsoe et al. 1982, Dahlberg and Stenstrom 1991, Danielson 1988, Lamb 1979, Thomas et al. 1983, Villeneuve et al. 1991).

Such "nursery-fungi", include *Thelephora terrestris* Pers., *Laccaria laccata* (Scop. ex Fr.) Cke., *Hebeloma crustuliniforme* and the "E-strain" fungi (*Wilcoxina mikolae* Yang & Korf and some other related ectendomycorrhizal species) (Chu-Chou and Grace 1987, 1983, 1981, Mason et al. 1983, Mikola 1989). Nursery fungi are "replaced" by naturally occurring ectomycorrhizal fungi that colonize new root growth. Persistence has been observed infrequently (e.g. Chu-Chou and Grace 1990, Grossnickle and Reid 1982). There has been little investigation of the reasons for replacement following outplanting, although differences between the soil environment in the nursery and the field have been implicated (Benecke and Gobl 1974, Castellano and Molina 1989, Chu-Chou and Grace 1990, Marx 1980, Perry et al. 1987).

Seedlings inoculated in the nursery with ectomycorrhizal fungi adapted to hot, dry soil conditions survived and performed better than uninoculated seedlings when planted on harsh sites in southwest Oregon (Castellano and Molina 1989, Theodorou and Bowen 1970). In each case the fungi applied continued to colonize the seedlings after outplanting. Ectomycorrhizas are more likely to persist when seedlings are planted on sites that lack appropriate ectomycorrhizal

fungi. Such sites may be those where exotic host species are planted, extremely hot and dry sites, or sites at which ectomycorrhizal hosts have not been growing for several years (Trofymow and van den Driessche 1991).

**3. Establishment of ectomycorrhizas on seedlings following clearcutting or fire.** Seedlings planted within a year after clearcutting form more ectomycorrhizas than if planting is delayed for several years (Amaranthus and Perry 1990), probably because inoculum levels of mycorrhizal fungi decline rapidly. Regeneration problems at hot, dry, disturbed sites may be due in part to an observed deficiency of ectomycorrhizas (Parke et al. 1984). Poor ectomycorrhizal colonization is related to low levels of inoculum and of organic matter to hold moisture and nutrients (Amaranthus and Perry 1990).

Whether planted or occurring naturally, seedlings on deforested areas can be infected by ectomycorrhizal fungi that have survived in the soil in various forms since the demise of a previous forest, or by fungi that have entered the soil since as air-borne spores (Ba et al. 1990, Lamb 1979).

Following clearcutting or fire, ectomycorrhizal fungi can survive in several ways: i) in senescent mycorrhizas; ii) in mycorrhizas on alternative hosts; iii) as resistant, dormant structures such as sclerotia or some types of spores and mycelium; and (perhaps), iv) saprotrophically.

Live ectomycorrhizas on the roots of cut trees have been found 9-18 months following cutting and probably do not survive more than a year in most cases (Harvey et al. 1980, Dahlberg and Stenstrom 1991). Harvey et al. (1980) recommended a fall or winter cut followed by planting of trees in spring to take advantage of residual ectomycorrhizas. Alternative hosts provide a continual source of inoculum. Many of the fungi that form ectomycorrhizas with members of the Pinaceae also do so with

plants of other families, including the Ericaceae, Fagaceae, Pyrolaceae, Rosaceae and Betulaceae (Amaranthus and Perry 1990). Important alternative hosts in Oregon clearcuts include species of *Arbutus*, *Lithocarpus* and *Arctostaphylos* (Perry et al. 1987). In a greenhouse bioassay, Douglas-fir seedlings formed ectomycorrhizas predominantly with *Rhizopogon* species and *Cenococcum geophilum* in mineral soil taken from beneath three hardwood species in a 5-year-old clearcut in southwest Oregon, but formed predominantly a brown type in soil from grassy areas nearby (Borchers and Perry 1990). The seedlings in "hardwood soil" were twice as large, perhaps because of the mycorrhizas, or perhaps because of levels of mineralizable nitrogen that were several times greater.

If many ectomycorrhizal fungi in mature stands are not actively symbiotic in young stands, what is their source of inoculum? Many ectomycorrhizal fungi can form resistant spores, sclerotia, or other resistant structures (Harley and Smith, 1983). Yet if a fungus found only in mature or older stands relies on survival in soil to reinfect new stands following clearcutting or fire, then it must produce extremely long-lived survival structures. More likely, such a fungus would depend on dispersal from older stands, or would survive in symbiosis with alternative hosts that survive the disturbance. Most spores and mycelial fragments survive less than two years, whereas continual inputs of spores and other propagules may maintain inoculum levels (Perry et al. 1987). Ectomycorrhizas are dispersed by wind from epigeous sporocarps (Marx and Kenny, 1982) and by rodents from hypogeous sporocarps (Maser 1978).

The saprophytic abilities of ectomycorrhizal fungi in nature are largely unknown, but could be important to their survival in the absence of a host. The ability of some ectomycorrhizal fungi to grow (albeit slowly) or use organic nitrogen sources in axenic and monoxenic (synthesis) culture

may indicate some saprophytic ability in nature.

#### 4. Succession of ectomycorrhizas as forests age.

Differences between the sporocarp flora of young and old forests have been recognized for many years (Last et al. 1983, Romell 1938, Trappe and Strand 1969). Table 2.1 lists some of the documented cases of ectomycorrhizal succession. Genera and species of fungi (and in some cases even isolates (Chu-Chou and Grace 1990)) can be grouped according to the stage(s) of stand development in which they most commonly form ectomycorrhizas. Thus, Table 2.2 generalizes data from Table 2.1 and other studies (Thomas et al. 1983, Villeneuve et al. 1991, Zak 1971). At other sites and forest types there are probably many exceptions to these generalizations. In young stands (< 12 yr) succession was directly observed, while succession in older stands was inferred from comparisons of stands of different ages. Succession appears to be most rapid in young stands, often with almost complete changes in the species composition of the ectomycorrhizal community (Danielson and Pruden 1989, Last et al. 1984, Mason et al. 1983). Thereafter, new ectomycorrhizas tend to appear occasionally and the relative abundance of ectomycorrhizas may change. Ectomycorrhizas that are dominant in young stands may be present in low numbers in older stands (Visser and Danielson 1990). *Elaphomyces muricatus* Fries and *Hysterangium crassirachis* were found in abundance in six old-growth (200-450-year-old) Douglas-fir forests in Oregon, but not in two mature (70,80-year-old) stands, during a survey of hypogeous sporocarps of known ectomycorrhizal fungi (Luoma et al. 1991). This casts some doubt on Meyer's (1973) suggestion that mycorrhizal conditions in Douglas-fir forests are probably not influenced significantly by stopping forest succession in the subclimax stage.

Present evidence indicates that the concept of ectomycorrhizal succession applies to even-aged stands only.

In uneven-aged stands, seedlings and mature trees whose root zones overlap have been observed to form ectomycorrhizas with the same species of late-stage fungi (Danielson and Pruden 1990, Fleming 1983, 1984, Griffiths et al. 1991, Thomas et al. 1983).

#### 5. Differences between early-stage and late-stage fungi.

Deacon et al. (1983) grew birch seedlings in soil taken from beneath mushrooms of several ectomycorrhizal fungi of a successional series. Early-stage fungi (*Laccaria*, *Hebeloma*, *Inocybe*) formed ectomycorrhizas in soil from under their sporocarps, *Lactarius* (intermediate-stage) rarely did and *Leccinum* (late-stage) did not colonize the seedlings. Instead, early-stage fungi formed ectomycorrhizas in soil from beneath *Lactarius* and *Leccinum* species. Seedlings were also sown into non-mycorrhizal but unsterile soil amended with inocula of mycorrhizal fungi grown in the laboratory in a peat and vermiculite mixture (Deacon et al. 1983). Again, *Hebeloma* and *Laccaria* were able to form mycorrhizas, some *Lactarius* mycorrhizas formed, but the late-stage fungi *Leccinum* and *Amanita* did not form mycorrhizas.

When soil was "seeded" with basidiospores, three *Hebeloma* species (early-stage fungi) formed ectomycorrhizas, but *Lactarius* and *Leccinum* did not (Fox 1983). These studies show the inability of late-stage fungi to form ectomycorrhizas in non-sterile greenhouse conditions. The inability of greenhouse bioassays to accurately reflect the presence of ectomycorrhizal inoculum in soils from forests (Parke et al. 1983, Pilz and Perry 1984) may have been due to the presence of late-stage fungi.

Under some conditions late-stage fungi will form ectomycorrhizas with seedlings. *Lactarius*, *Leccinum scabrum* (Bull.: Fr.) S.F. Gray and *Leccinum roseofracta* Watl., but neither *Russula* nor *Cortinari*, formed ectomycorrhizas with non-mycorrhizal birch seedlings when they were planted with minimal disturbance in soil containing ectomycorrhizas of

these fungi (Fleming 1983, 1984). Where the planting locations were "isolated" by cutting or trenching, only early-stage mycorrhizas developed.

In monoxenic culture, ectomycorrhizas of late-stage fungi such as *Amanita muscaria* and *Leccinum scabrum* were readily synthesized, but did not persist when the seedlings were planted in non-sterile soil (Fleming 1985, Mason et al. 1983). Thus, late-stage fungi seem to be at a competitive disadvantage unless connected to a host beyond the seedling stage, regardless of the amounts of inoculum present. Note that the terms "early-stage" and "late-stage" as used by Deacon et al. (1983), Fleming (1983, 1984) and Mason et al. (1983) refer to a succession of fungi in a plantation of birch only 12 years old established on old agricultural land. Distinct mechanisms may be responsible for succession in older forests. Naturally occurring seedlings in forests often form ectomycorrhizas with late-stage fungi and are in most cases probably linked to the dominant trees by mycelia (Griffiths et al. 1991b). Just as inoculum levels do not explain the absence of late-stage ectomycorrhizas in young plantations, the paucity of early-stage ectomycorrhizas in mature forests is due to other factors, as soil in mature forests contains propagules of early-stage fungi (Dahlberg and Stenstrom 1991, Parke et al. 1983, Pilz and Perry 1984).

The succession of ectomycorrhizas on birches has a spatial pattern easily seen in the position of mushrooms of the ectomycorrhizal fungi. As the tree grows, early-stage ectomycorrhizas do not disappear, but form progressively larger rings centred at the bole, while the late-stage ectomycorrhizas appear first near the bole and move outwards as well (Ford et al. 1980, Mason et al. 1982). This pattern may be related to the changes in the soil due to mycorrhizal activity and litter accumulation. In a young eucalypt plantation, late-stage ectomycorrhizal fungi produced sporocarps where litter had accumulated in linear

depressions left by site preparation (Gardner and Malajczuk 1985). Differences in the chemistry of litter of different conifers lead to distinct environments in the forest floor, which may help explain why late-stage fungi generally have a narrower host-range than early-stage fungi (Malajczuk et al. 1982).

Many ectomycorrhizal fungi can use peptides, peptones and proteins as a sole nitrogen source when cultured *in vitro*, an ability that may allow mycorrhizal plants to access otherwise unavailable nitrogen sources in decomposing litter (Abuzinadah et al. 1986, Abuzinadah and Read 1989, 1989a, 1986, 1986a). Finlay and Frostegard (1990) found that the late-stage fungi *Suillus variegatus* (Swartz: Fr.) Kuntze, *Piloderma croceum* and other unidentified isolates had a greater capability to degrade and use proteins *in vitro* than the early-stage fungi *Laccaria laccata*, *L. proxima* (Boud.) Pat, *Hebeloma crustuliniforme* and *Thelephora terrestris*. Observing the production of fatty-acid esterase by late-stage fungi that form ectomycorrhizal mats, Caldwell et al. (1990) hypothesized that lipids might supply the high energy requirements of late-stage fungi that produce enzymes to degrade lignin and other complex nitrogen sources.

#### **6. Possible explanations of ectomycorrhizal succession.**

As an ectomycorrhizal root tip grows, the mycosymbiont must keep pace or else leave the root tip open for colonization by another fungus. Replacements of one ectomycorrhizal fungus by another on a root tend to occur when root growth resumes following dormant periods during cold or drought (Marks and Foster 1967, Fleming 1985, Fleming et al. 1984, Wilcox 1968). But why do ectomycorrhizas fail to maintain themselves and what determines the order of a succession of ectomycorrhizas? Succession of species of understory plants in Douglas-fir forests is apparently determined by their mechanisms for survival and dispersal, as well as changes in soil and microclimate conditions (Halpern 1988). The same

factors probably play a role in ectomycorrhizal succession, with the additional complication of changes in host physiology and rooting as forests age. Colonization and modification of substrates by saprotrophic fungi can lead to successions (Harper and Webster 1964, Moser 1949, Rayner and Boddy 1988). Mason et al. (1987) suggested that early-stage ectomycorrhizal fungi tend, like those fungi responsible for the early-stages of wood decay, to be adapted for rapid growth and exploitation of readily metabolized nutrient sources; while late-stage ectomycorrhizal fungi and the fungi that colonize wood in an advanced state of decay tend to be slower-growing but better able to survive in less favourable conditions.

Organic matter accumulation may be one of the changes in the soil environment that drive ectomycorrhizal succession (Gardner and Malajczuk 1985, Mason et al. 1982, Visser and Danielson 1990). As stands age, there is also a net movement of nutrients, especially nitrogen, from the mineral soil to the forest floor (organic layer), accompanied by a parallel redistribution of feeder roots (Grier et al. 1981, Gessel et al. 1973). Yet there is no experimental evidence to support the speculation (Chu-Chou and Grace 1990, Fleming et al. 1984, Last et al. 1983, Thomas et al. 1983) that changes in the soil environment are a major cause of ectomycorrhizal succession. That seedlings form primarily early-stage ectomycorrhizas in greenhouse bioassays of soil from mature forests could be seen as evidence that soil factors are not important to succession. However, the nutrient status and biological activity of the soil samples used probably changed considerably as a result of root death, physical disturbance and temperature and moisture changes. Cutting roots could increase nutrient availability significantly due to elimination of uptake and decomposition of roots and mycorrhizas. A similar argument could be made about the tendency for trap-seedlings to form early-stage

ectomycorrhizas when planted in "isolated" soil (around which roots were cut) in forests (Fleming 1984). Large hosts may provide late-stage ectomycorrhizal fungi with a large energy source needed for infection of seedlings. If succession is to be explained on the basis of a combination of soil nutrient status, enzymatic capabilities of the fungus, and host physiology, then a model is needed that considers the costs and benefits to each symbiont. For a host that is limited in its growth more by photosynthate than by nutrient supply, perhaps an early-stage fungus is the appropriate symbiont. Conversely, a late-stage fungus may be of more benefit to a host limited more by nutrients than photosynthate.

Other influences on ectomycorrhizal succession have been suggested or implicated. Fleming (1985) found that the initial ectomycorrhiza established on a seedling has some influence on which ectomycorrhizas followed. The importance of rhizosphere organisms has been suggested, but not investigated (Fleming et al. 1984, Last et al. 1983). In Australia, naturally regenerating *Pinus radiata* D. Donn and *P. elliottii* Engelm. seedlings that were more than 800 m from mature stands formed ectomycorrhizas only with species of fungi that were producing sporocarps in the mature stands (Lamb 1979). In this case and in New Zealand plantations of exotic trees (Chu-Chou and Grace 1987), lack of inoculum appears to influence the successions observed. Any explanation of ectomycorrhizal succession should also account for the ability of fungi such as *Cenococcum geophilum* and *Rhizopogon vinicolor* to be dominant mycorrhiza formers in stands of all ages. Interestingly, both are known for their ability to survive and function in relatively dry soils (Mexal and Reid 1973, Perry et al. 1987, Piggot 1982), which may give them a competitive advantage in soils of varying fertility and allow them to survive periods of drought or freezing.

More information is needed about the mycorrhizal status and soil conditions of naturally occurring forests of various ages, in order to gauge the effect of soil variables. It would be especially useful to know the role that different ectomycorrhizal fungi play in the soil. What substrates do they exploit and under what environmental conditions? With such ecological knowledge, conservation of ectomycorrhizas and their functions could more easily be considered when choosing forestry practices.

## Methods and materials

**1. Selection of study sites.** Potential sites were identified on forest-cover-type maps and the mature (80-90-yr-old) and old-growth stands (>250 yr) of each site were traversed on foot. Two sites were selected, each with an old-growth and a mature stand well-matched in species composition, soil, slope, aspect and topography. Stand ages were determined from increment cores of 8 dominant or codominant trees in each stand. Although mature stands of logging origin were sought, these were located at lower elevations than the remaining old growth. It was therefore necessary to compare old growth with mature stands that regenerated following stand-destroying fire. The Koksilah site was selected by Dr. J.A. Trofymow as a part of the Coastal Forest Chronosequence project by the Canadian Forest Service. Several studies of other groups of organisms and ecological processes have been made at the Koksilah chronosequence (Marshall 1993).

**2. Description of sites studied.** The two sites and two stands at each site are described in detail in Appendix 2A. Both sites were on southeastern Vancouver Island in the Very Dry Maritime subzone of the Coastal Western Hemlock biogeoclimatic zone (CWHxm) (Green and Klinka 1994), within 40 Km of Victoria, British Columbia. The first site studied was several kilometres north of the Koksilah River. The second was a kilometre southeast of Goldstream Lake. A single 60 m by 60 m square plot was studied in each of the four stands, two old growth and two mature. Each plot had a well-drained Brunisolic soil, moderate slope and a southerly exposure. Douglas-fir, *Pseudotsuga menziesii*, dominated each stand. Western hemlock, *Tsuga heterophylla* (Raf.) Sarg. and western red cedar, *Thuja plicata* (Donn ex D. Don), accounted for less than 20% of the dominant or co-dominant trees in each of the four plots. The mature stands were 76% and 68%

as tall (maximum height) as old growth at Koksilah and Goldstream respectively and trees were 39% and 51% as large (maximum diameter at breast-height). At each site there was a patchy distribution of abundant suppressed Douglas-fir (10-15 cm mean dbh and 8-13 m mean height), especially in the old-growth plots. *Gaultheria shallon* Pursh (salal) dominated the understory vegetation in all plots. None of the plots had been affected by any logging.

Species of understory vegetation indicate a drier soil environment at Koksilah than at Goldstream. *Holodiscus discolor* (Pursh) Maxim., absent from the site at Goldstream, was a codominant understory shrub at Koksilah; and cedar, hemlock and red huckleberry (*Vaccinium parvifolium* Sm.) were less frequent at Koksilah. The Koksilah site is near the top of a slope, is steeper, is more exposed to wind and was classified as a poor quality site (slow growth of tree height). The Goldstream site is near the bottom of a slope, less than a kilometre from Goldstream Creek and was classified as a medium quality site.

Seventy-five percent of the soil substrate by area was LFH materials over mineral soil. Almost all decayed wood was covered by LFH materials, and bedrock covered by LFH was present in all plots. The frequency of substrate types was very similar in old-growth and mature plots within each site. At Goldstream the forest floor (L+F+H) and the F and H layers were deeper in the old-growth than in the mature plot, but there were no significant differences in LFH depths at Koksilah. At both Koksilah and Goldstream the humus layer in both plots was predominantly humifibrimor, with some moder. At each site, well-decayed logs over 16 cm diameter made up 95% of the volume of coarse woody debris, and about twice as much coarse woody debris was present in old-growth as in mature plots. Logs of 40 cm diameter or larger were common in old growth and virtually absent from mature plots.

**3. Sampling design.** A stratified, randomized sampling design was used. At each site, an identical design with separate randomizations was used for the two plots, mature and old growth. Sampling was stratified according to five broad soil habitat classes: decayed logs, decayed stumps (bases of broken stems rather than cut stems), LFH over bedrock or gravel, LFH and mineral soil at the base of codominant trees, and LFH and mineral soil elsewhere. Stratification was used to allow comparison of the ectomycorrhizal communities of these habitats. In combination with a survey of the amounts and locations of each of these habitats within each plot, stratification also allowed computation of the variance of ectomycorrhizal parameters for a plot as a whole. At Koksilah, 72 soil cores were taken and analyzed for numbers and types of ectomycorrhizas, 36 from each age class (plot). At each plot and at each of the three sampling times, 3 cores were taken from logs, 2 from stumps, 2 from near boles, 2 from rocky areas and 3 from other locations. Koksilah was sampled in May of 1992 and 1997 and in November of 1992. A map of each plot was divided into a grid of metre squares, supplying  $60 \times 60 = 3600$  possible sampling points. At Koksilah, each point ( $1 \text{ m}^2$ ) was assigned a habitat type based on a sketch map of the location of the habitats. Thus for each habitat a sequence of potential sampling points was numbered and used to draw a random sample. All stumps within each plot at Koksilah were also mapped and numbered to allow a randomized sample. Stumps were surveyed separately as a part of another study (Setälä and Marshall 1994). An objective of this study was to determine whether logs, stumps and soil near boles contained ectomycorrhizas different from those in soil elsewhere. In order to obtain enough data these habitats were sampled more intensively than they would have been by an unstratified design or by proportional representation. Measuring tapes were used to

locate the sampling points specified by a pair of coordinates. Rules were devised to locate a sampling point on a log objectively if the coordinates did not land on a log. Samples from stumps were taken vertically starting at the top and included any bark remaining. Samples near boles of living trees were taken 5 cm from the bole of codominant mature trees and 15 cm from the bole of old-growth trees, the nearest that cores could be taken without hitting the outward sloping butts of the trees. Samples from stumps and near boles were taken on the north, east, south or west sides according to whether the sampling coordinates were both even, east-west was even and north-south was odd, east-west was odd and north-south was even, or both odd, respectively. A provision was made during randomization to ensure that all sampling points were separated by 2 m or more.

Forty-eight cores were extracted at Goldstream, 24 in November of 1993 and 24 in May of 1994. Because only 12 core samples could be processed per plot each season, insufficient data were obtained at Koksilah to calculate useful estimates of variances of ectomycorrhizal parameters within habitats and thereby of each plot as a whole. Consequently, a simplified scheme was used at Goldstream. The habitats were not mapped. Instead, each sampling point was found by starting at a random coordinate and following a random bearing if necessary to find suitable habitat. Equal numbers of samples were collected from each habitat. The same habitats were sampled as at Koksilah, except that the forest floor on rocky areas was not sampled at Goldstream because there was little of this habitat in the old-growth plot. To avoid an influence of sampling time on the comparison of mature and old-growth stands, equal numbers of samples were taken and the same habitats were sampled from each age class on a sampling day.

Location of 24 sampling points, extraction of 24 cores

and the recording of notes on the sampling points took 1-2 days in the field. Cores were given a serial number and labelled with their habitat, but not the age class. At the time of its examination I was not aware of the age class from which a core sample had been taken. Sampling point parameters measured were slope, aspect, species of vegetation within 1 m, height of salal, distance to nearest dominant or codominant tree, species and diameter of nearest dominant or codominant tree, thickness of the L, F and H layers, depth of the core, and presence of any fungal sporocarps within 2 m. Methods of sampling, storage of soil, extraction of ectomycorrhizas from soil, separation of types within a soil sample and description of types, are described in chapter one.

**4. Statistical analysis.** Analysis of variance was done with PROC GLM of SAS Institute Inc. software (SAS Institute 1985). Normality of residuals was tested using PROC UNIVARIATE. Homogeneity of variance was tested with scatter plots of residuals versus predicted values and standard deviations versus means for each value of the classes in the model. Logarithmic transforms were applied when necessary for the data to satisfy the assumptions of the statistical model. Chi-squared tests of goodness of fit and t-tests for means comparison were performed according to the methods of Zar (1984).

## Results

At both Koksilah and Goldstream more ectomycorrhizal root tips were observed in old growth than in the mature stands (5% and 23% respectively) (Table 2.3), but these differences were not statistically significant. Probability values were .69 and .38, respectively, in a paired t-test with habitats as replicates; and were greater than .25 according to an analysis of variance with a full model (Tables 2.4 and 2.5).

Almost identical numbers of types were found in old-growth and mature stands, at each site and overall (Table 2.3). At each site, the rate at which new types were encountered was very similar in the two age classes (Fig. 2.1). The shapes of these progress curves do not suggest any difference in richness of types between the two age classes. Plots of logarithms of abundances of species versus descending ranks according to abundance are termed equitability plots as they display the extent to which species or types have similar abundances. For the types of this study they are sigmoid and indicate very similar diversity of mycorrhizal types in old-growth and mature stands (Fig. 2.2).

Sufficient data of the frequency of observation (number of core samples in which a type was found) of types were obtained to allow a Chi-square goodness of fit comparison of the age classes for the nine most frequent types at both sites (Fig. 2.3). The test provided some evidence ( $p=.09$ ) that the frequency of types is not independent of age class, although these observations are not independent; therefore the true probability is greater. The abundance (number of root tips of a type) of these types was also compared (Figs. 2.4, 2.5). At each site, *Cenococcum geophilum* (type 1), *Hysterangium* (type 2) and type 16 were more frequent and more abundant in mature than in old-growth stands and,

conversely, *Rhizopogon vinicolor* was more frequent and abundant in the old growth than in the mature stands. Analysis of variance of abundance data for Koksilah and Goldstream (Tables 2.4, 2.5) gives marginal evidence ( $p=0.2$ ) and some evidence ( $p=0.07$ ), respectively, that some of the most frequent types are more abundant in one stand than the other (but note pseudoreplication). As Figure 2.5 shows, the variability relative to the mean is large for each type.

Of the 21 types that are common or distinctive (class a) but rank 10th or lower in frequency, all but 5 are represented in both age classes (Fig 2.6). Taken on their own, there is some statistical significance ( $p=.03$ ,  $p=.06$  by Fischer's exact test) to the observations of types 43 and 65 in 4-5 cores each from old growth and their absence in the samples from mature stands. However, two such cases out of 21 would not be unexpected under a null hypothesis that the distribution of types is independent of age class. There is no evidence that any types were specific to mature stands.

## Discussion

Suitable sites with matched pairs of mature and old-growth stands of Douglas-fir were selected after 2-3 weeks of field work and rejection of several sites. The progression of logging from more productive and more easily accessible sites at low elevation to less fertile higher elevation sites has left old-growth remnants in the upper parts of drainages, and mature stands of logging origin in the valley bottoms. The central question to be addressed by this study was whether mature second-growth stands can provide habitat for the ectomycorrhizas found in old growth. Since all or most mature second-growth stands in a managed forest will have regenerated following logging and since there may be differences between stands of logging and fire origin, stands of logging origin were sought. Matched pairs of old-growth and mature stands of logging origin were not found, leading me to study mature stands of fire origin.

Geographical separation of the age classes also prevented an alternative study design in which ten or more stands of each age class were sampled less intensively. Rather than matching individual stands, a group of old-growth stands could be compared to a group of mature stands that is similar overall in topography, drainage and other features not determined by the disturbance event that lead to the establishment of the mature stands. Conclusions based on data from many sites are more likely to apply to the biogeoclimatic subzone as a whole. Such an approach might be feasible and preferable in zones with a different logging history. Conversely, an advantage of studying only two sites was that it was possible to describe them in detail. Detailed knowledge of the ecosystems of the sites of this and other studies may reveal a relationship between the ectomycorrhizal communities and their environments. Also, more variability would be expected if many sites were

sampled.

Conclusions about differences between age classes are tentative. Probability values for comparisons of age classes based on data of both sites pooled are best viewed with caution, as they are based on pseudoreplicates--the observations within a plot or site are not independent. Statistical differences between mature and old-growth plots at a site may be due to the age of the stands, or to other differences between the plots. Each of the two sites in this study gave a replicate of the age class comparison--it remains to future studies to provide more replication. The study was not designed to detect differences between sites. Site and season are confounded factors. Nor was it designed to estimate parameters for the plots surveyed, but rather to compare age classes and habitats. Means of parameters based on ectomycorrhizal sampling are not estimates of true means for the plots, but are specific to the sampling design used, as logs, stumps and soil near boles were sampled more intensively than they would have been by proportional representation.

Disturbance of an ecosystem by fire can be similar in many ways to that caused by clearcutting and slash burning. Therefore the results of this study probably can be applied to many logged sites, even though mature stands of fire origin were studied. On the other hand, if clearcutting is accompanied by severe soil disturbance or compaction, or abundant slash is left unburnt, or if most of the coarse woody debris is removed for utilization, then there may be important effects on mycorrhizas that would not be seen in most stands of fire origin. Both fires and harvesting vary greatly in their effects on soil, making generalizations difficult.

Non-parametric diversity indices such as the Shannon-Weiner tend to be correlated with richness (number of species) (Ricklefs 1979) and are not based on models of the

structure or function of the community. Plots of species—versus observations and plots of abundance versus rank indicate richness and equitability (degree to which species are similar in abundance), the components of diversity. The sigmoid equitability curves of logs of ectomycorrhizal abundance versus rank (Figs. 2.2) best fit the log normal distribution or perhaps MacArthur's broken stick model (Southwood 1978). Neither the log normal nor the broken stick model gave a reasonable estimate of richness of types, probably because the data did not adequately fit either model. Old-growth and mature stands showed almost no difference in both progress curves and equitability curves, indicating very similar richness, equitability and diversity of types.

A chi-square goodness-of-fit test and analysis of variance gave some evidence that abundances of the most common types were dependent on the age class of the stands. Several types were more abundant in one age class than the other at each site. A chi-square test of independence of age class and type using data of both sites pooled was performed as there were insufficient frequency data to test by site, although observations within a site are not independent. A pooled analysis of variance was not computed because the resulting pseudoreplication would have impeded interpretation.

The only other study comparing ectomycorrhizal fungi of old-growth and mature forests of Douglas-fir (Luoma et al., 1991) also found similar communities, with some differences that were not statistically evaluated. My observation of more *Rhizopogon vinicolor* ectomycorrhizas in old-growth than in mature stands is not consistent with Luoma et al.'s (1991) observation that this species formed hypogeous sporocarps as abundantly in mature stands as old-growth stands of Douglas-fir in Oregon. Several other types appeared to be more abundant in one age class than the

other, but as it is statistically invalid to test hypotheses formed a posteriori, additional study is required to statistically confirm or deny these trends.

Most old-growth and mature second-growth forests of Douglas-fir probably have similar communities of ectomycorrhizas. Before the importance of old-growth Douglas-fir stands to conservation of the diversity of ectomycorrhizal fungi can be assessed, investigation into the distribution of less common ectomycorrhizal fungi is needed at sites with different frequencies and intensities of disturbance.

**Tables and figures**

**Table 2.1. Some cases of ectomycorrhizal succession**

Location	Reference	Host	Fungus	Stand Ages <sup>a</sup>
New Zealand	Chu-Chou and Grace 1990	<i>Pinus radiata</i>	<i>Suillus</i>	<5
			<i>Rhizopogon rubescens</i> Tul.	5-10 <sup>b</sup>
	Chu-Chou 1979	<i>Pinus radiata</i>	<i>Hebeloma crustiliniforme</i>	nursery
			<i>Thelephora terrestris</i>	nursery
			<i>Suillus</i>	<5
			<i>Rhizopogon</i>	<20
			<i>Amanita muscaria</i>	14-50 <sup>b</sup>
	Chu-Chou and Grace 1988	<i>Pinus radiata</i>	<i>Amanita muscaria</i>	10-50 <sup>b</sup>
			<i>Tricholoma pessundatum</i> (Fr. Quél.	10-50 <sup>b</sup>
	Chu-Chou and Grace 1981	<i>Pseudotsuga menziesii</i>	<i>Hebeloma crustiliniforme</i>	nursery
			<i>Rhizopogon vinicolor</i>	0-50 <sup>b</sup>
			<i>Amanita muscaria</i>	12-50 <sup>b</sup>
	Chu-Chou and Grace 1983	<i>Pseudotsuga menziesii</i>	<i>Laccaria laccata</i>	nursery
			<i>Thelephora terrestris</i>	nursery
			<i>Tuber</i>	<5
			<i>Endogone flammicorona</i> Trappe & Gerdemann	<5
			<i>Amanita muscaria</i>	13-75 <sup>b</sup>
	Chu-Chou and Grace 1987	<i>Pseudotsuga menziesii</i>	<i>Hebeloma crustiliniforme</i>	nursery
			<i>Endogone flammicorona</i>	nursery
			<i>Laccaria laccata</i>	nursery
<i>Rhizopogon parksii</i> A.H. Smith			0-40 <sup>b</sup>	
<i>Suillus lakei</i> (Murr.) A.H.S. & Thiers			0-40 <sup>b</sup>	

Location	Reference	Host	Fungus	Stand Ages <sup>a</sup>
New Zealand (cont.)	Chu-Chou and Grace 1982	<i>Eucalyptus</i> spp.	<i>Scleroderma</i>	nursery
			<i>Hydnangium carneum</i>	5-50 <sup>b</sup>
			unidentified basidiomycete	30-50 <sup>b</sup>
Australia	Gardener and Malajczuk 1985	<i>Eucalyptus</i> spp.	<i>Pisolithus</i>	0-3
			<i>Scleroderma</i>	0-5
			<i>Laccaria</i>	0-5
			<i>Amanita</i>	5-?
			<i>Cortinarius</i>	7-100 <sup>b</sup>
			<i>Paxillus</i>	7-100 <sup>b</sup>
			<i>Ramaria</i>	?-100 <sup>b</sup>
			<i>Russula</i>	?-100 <sup>b</sup>
Alberta	Danielson 1984	<i>Pinus banksiana</i>	<i>Laccaria proxima</i>	5
			<i>Scleroderma macrorrhizon</i>	5
			<i>Astraeus hygrometricus</i> (Pers.) Morg.	5
			<i>Lactarius paradoxus</i>	5
			<i>Elaphomyces</i>	80
			<i>Suillus</i>	80
			<i>Cortinarius</i>	80
			<i>Cantharellus</i>	80
	<i>Hydnum</i>	80		
	Visser and Danielson 1990	<i>Pinus banksiana</i>	<i>Suillus brevipes</i> (Pk.) Kuntze	10
			<i>Inocybe</i> spp.	10
			<i>Coltricia perennis</i>	10
			<i>Astraeus hygrometricus</i> (Pers.) Morg.	10

Location	Reference	Host	Fungus	Stand Ages <sup>a</sup>
Alberta (cont.)	Visser and Danielson 1990 (continued)	<i>Pinus banksiana</i>	<i>Scleroderma macrorhizon</i>	10
			<i>Laccaria proxima</i>	10
			<i>Lactarius</i> spp.	10
			<i>Suillus tomentosus</i> (Kauff.) Sing., Snell & Dick	>40
			<i>Tricholoma</i> spp.	>40
			<i>Cortinarius</i> spp.	>40
			<i>Russula</i> spp.	>40
			Hydnaceae	>40
Scotland	Mason et al. 1982, 1983	<i>Betula</i> spp.	<i>Hebeloma crustiliniforme</i>	0-10 <sup>b</sup>
			<i>Laccaria</i>	0-10 <sup>b</sup>
			<i>Thelephora terrestris</i>	3-10 <sup>b</sup>
			<i>Inocybe</i>	4-10 <sup>b</sup>
			<i>Lactarius</i>	4-10 <sup>b</sup>
			<i>Hebeloma</i>	5-10 <sup>b</sup>
			<i>Cortinarius</i>	6-10 <sup>b</sup>
			<i>Leccinum</i>	6-10 <sup>b</sup>
<i>Russula</i>	10-10 <sup>b</sup>			

a Ages of Stands in which each ectomycorrhiza was most commonly found

b oldest stand studied

**Table 2.2. Successional position of some ectomycorrhizal fungi**

<b>Nursery Fungi:</b>	<i>Hebeloma crustiliniforme</i> <i>Thelephora terrestris</i> <i>Laccaria laccata</i>
<b>Early-stage Fungi:</b> ( $\leq 10$ yr)	<i>Hebeloma crustiliniforme</i> <i>Scleroderma</i> <i>Laccaria</i> <i>Wilcoxina</i> <i>Inocybe</i>
<b>Late-Stage Fungi:</b> ( $> 10$ yr)	<i>Cortinarius</i> <i>Leccinum</i> <i>Amanita</i> <i>Russula</i> <i>Cantharellus</i> <i>Hydnaceae</i>
<b>All-Stage Fungi:</b>	<i>Rhizopogon vinicolor</i> <i>Cenococcum geophilum</i>

**Table 2.3. Amount of soil sampled and abundance of ectomycorrhizal root tips and types in the four study plots. Stand ages, followed by standard deviations, are based on increment cores from eight dominant or codominant trees in each plot.**

site	Koksilah		Goldstream		both sites	
	mature 87 ± 6 yr	old growth 288 ± 45 yr	mature 89 ± 4 yrs	old growth 441 ± 8 yr	mature	old growth
ectomycorrhizal root tips	5,460	5,710	2,810	3,470	8,270	9,180
volume of soil sampled (cubic cm)	4,300	4,000	2,960	3,030	7,260	7,040
cores of soil sampled	36	36	24	24	60	60
ectomycorrhizal types	35	36	35	35	55	53

**Table 2.4. Analysis of variance of number of root tips at the Koksilah site colonized by the nine most frequent types of ectomycorrhizas. A logarithmic transform was applied to the data (sums of root tips colonized in three soil cores).**

<b>Source</b>	<b>DF</b>	<b>MS</b>	<b>F</b>	<b>F</b>	<b>Prob &gt; F</b>
<b>A: plot (fixed)</b>	<b>1</b>	<b>1.5400</b>	<b>MS A/MS AC</b>	<b>0.767</b>	<b>&gt; .25</b>
<b>B: habitat (fixed)</b>	<b>4</b>	<b>1.8400</b>	<b>MS B/MS BC</b>	<b>6.100</b>	<b>.001</b>
<b>C: type (random)</b>	<b>8</b>	<b>7.7400</b>	<b>MS C/MS error</b>	<b>9.310</b>	<b>&lt; .0001</b>
<b>AB: plot * habitat</b>	<b>4</b>	<b>2.8800</b>	<b>MS AB/MS ABC</b>	<b>46.020</b>	<b>&lt; .0001</b>
<b>AC: plot * type</b>	<b>8</b>	<b>2.0100</b>	<b>MS AC/MS error</b>	<b>2.420</b>	<b>.2</b>
<b>BC: habitat * type</b>	<b>32</b>	<b>0.3010</b>	<b>MS BC/MS error</b>	<b>0.362</b>	<b>&gt; .25</b>
<b>ABC: plot*hab*type</b>	<b>32</b>	<b>0.0626</b>	<b>MS ABC/MS error</b>	<b>0.075</b>	<b>&gt; .25</b>
<b>error</b>	<b>126</b>	<b>0.8310</b>	<b>Koksilah site</b>	<b>prob &gt; F (model) = .0001</b> <b>R squared= 55%</b>	<b>C.V.= 30%</b>

**Table 2.5. For the Goldstream site, analysis of variance of the number of root tips colonized by the nine most common types of ectomycorrhizas overall. A logarithmic transform was applied to the data (sums of root tips colonized in three soil cores).**

Source	DF	MS	F	F	Prob > F
A: plot (fixed)	1	1.270	MS A/MS AC	1.301	> .25
B: habitat (fixed)	3	2.500	MS B/MS BC	1.380	> .25
C: type (random)	8	6.290	MS C/MS error	12.630	< .0005
AB: plot * habitat	3	0.866	MS AB/MS ABC	2.560	.08
AC: plot * type	8	0.969	MS AC/MS error	1.945	.07
BC: habitat * type	24	1.804	MS BC/MS error	3.620	< .0005
ABC: plot*hab*type	24	0.338	MS ABC/MS error	0.692	> .25
error	72	0.498	Goldstream site	prob > F (model) = .002 R squared = 74% C.V. = 24%	

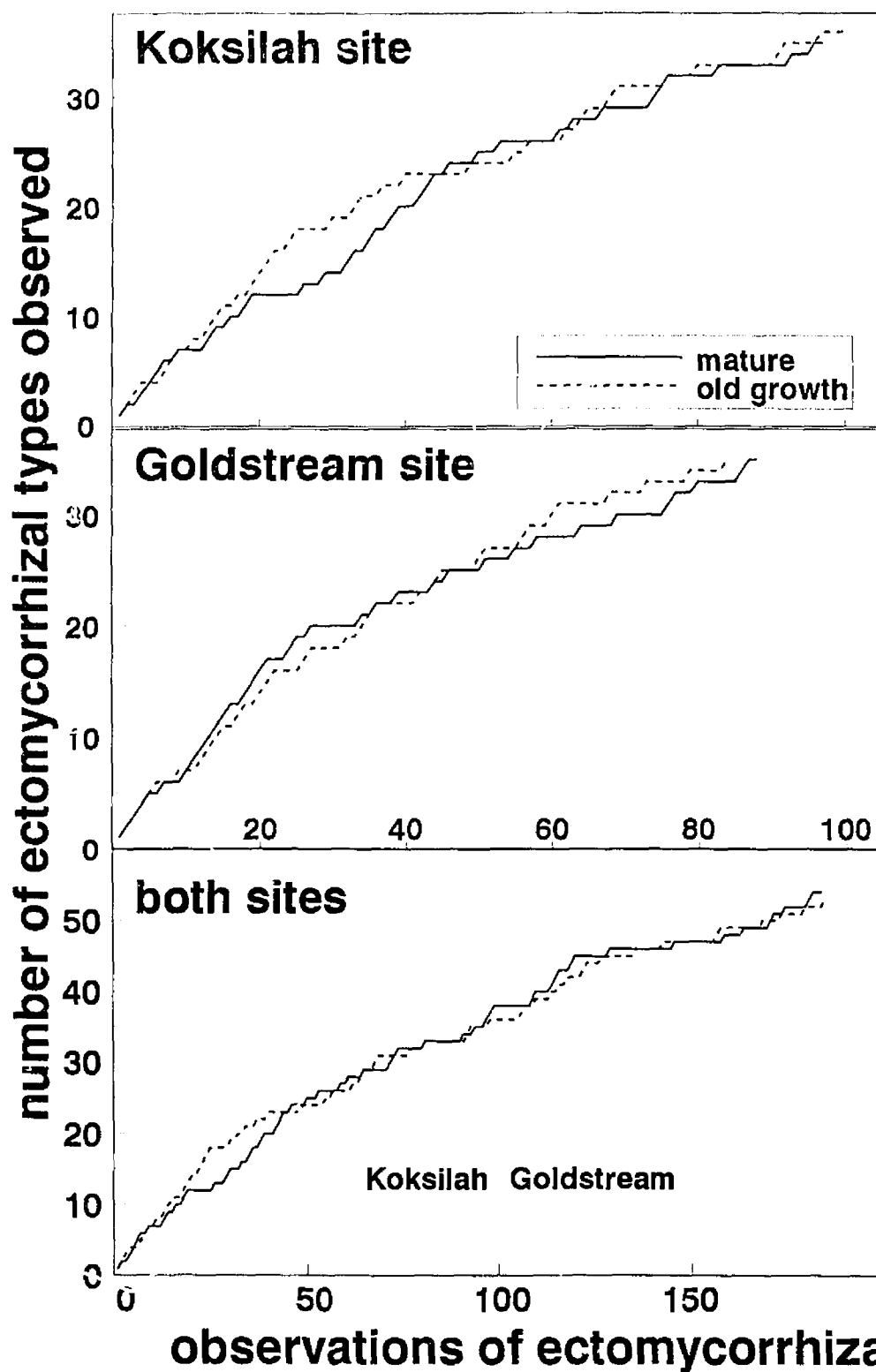
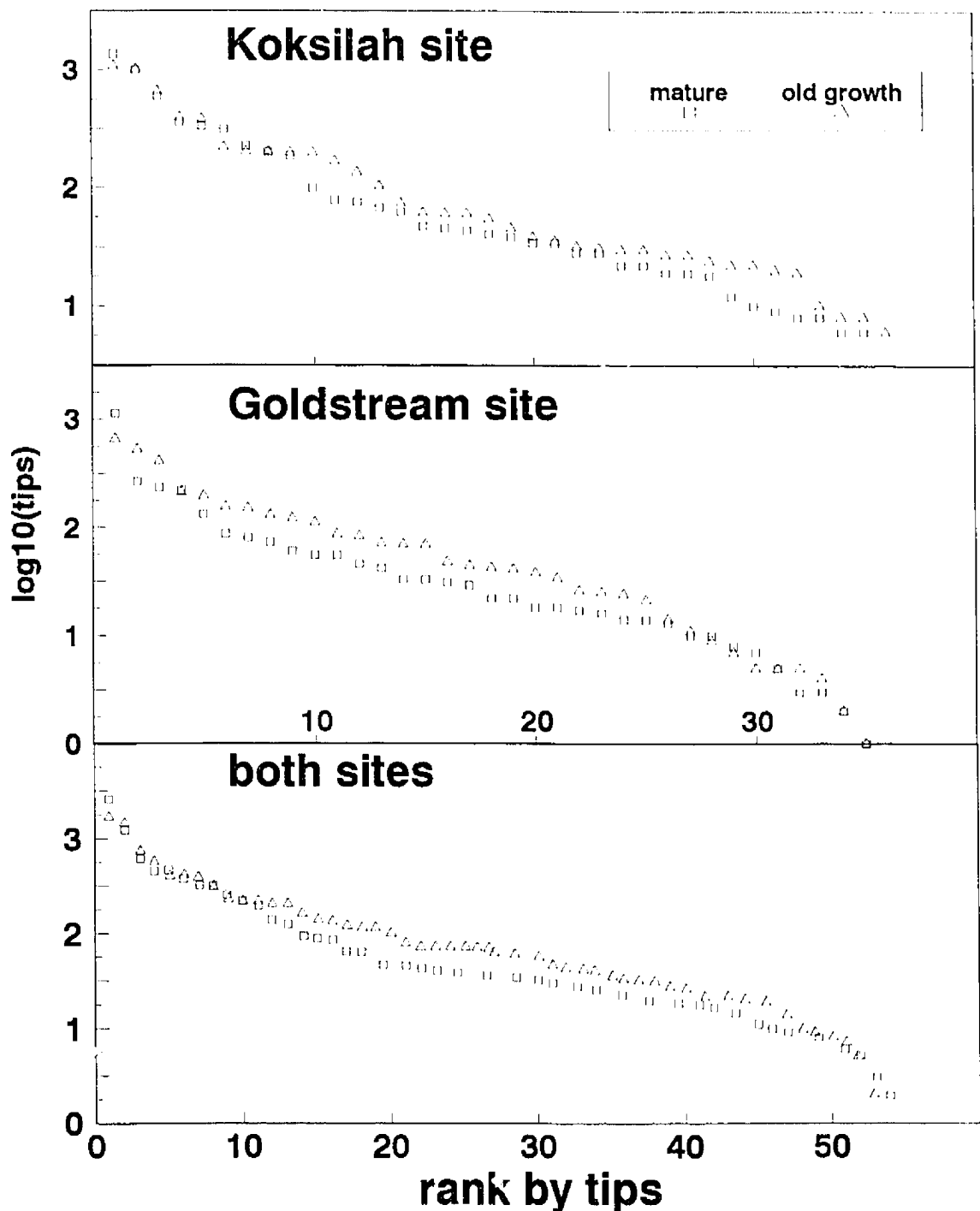
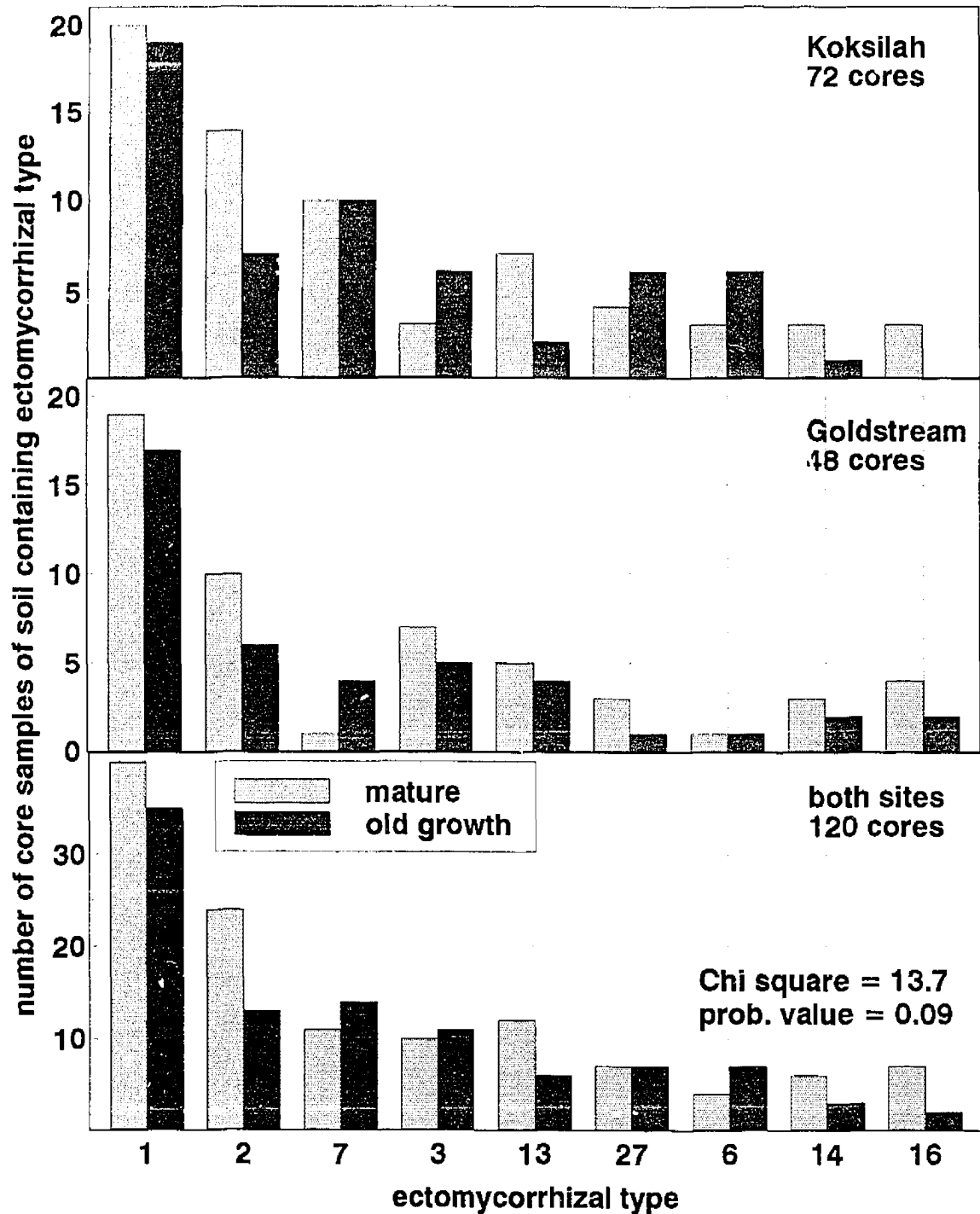


Figure 2.1. Progress assessing richness of ectomycorrhizal types in mature and old growth stands of Douglas-fir at each site. An observation is the occurrence of a type in a core sample of soil.



**Figure 2.2. Equitability curves for the four stands sampled--mature and old growth at two sites. Common logarithms of the number of root tips colonized by each ectomycorrhizal type as a function of its rank according to that abundance.**



**Figure 2.3. Frequency of the nine most frequent ectomycorrhizal types in mature and old growth stands at two sites.**  
**1 = *Cenococcum geophilum*, 2 = *Hysterangium*?, 7 = *Rhizopogon vinicolor*, 3 = *Piloderma*, 13 = *Lactarius deliciosus***

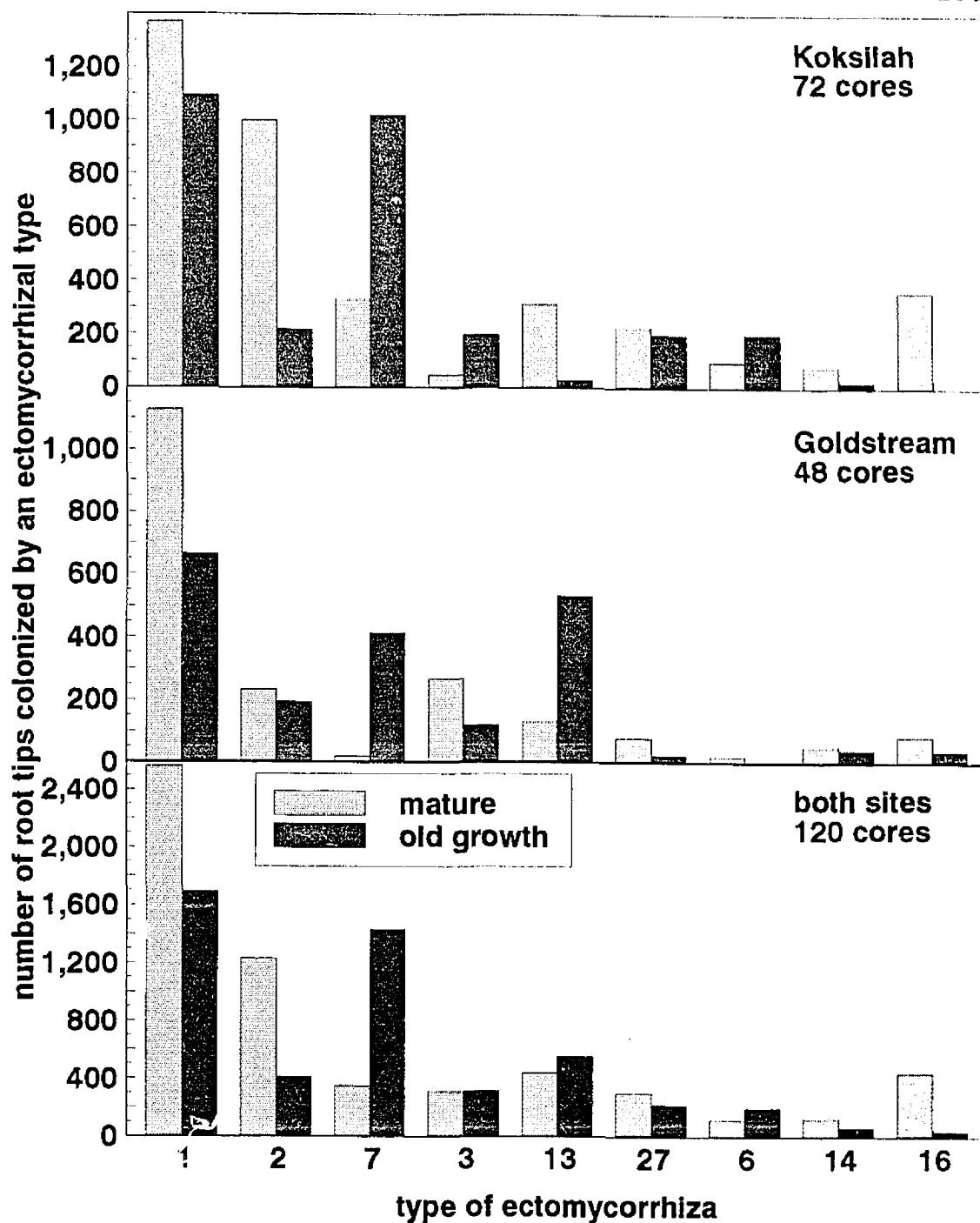
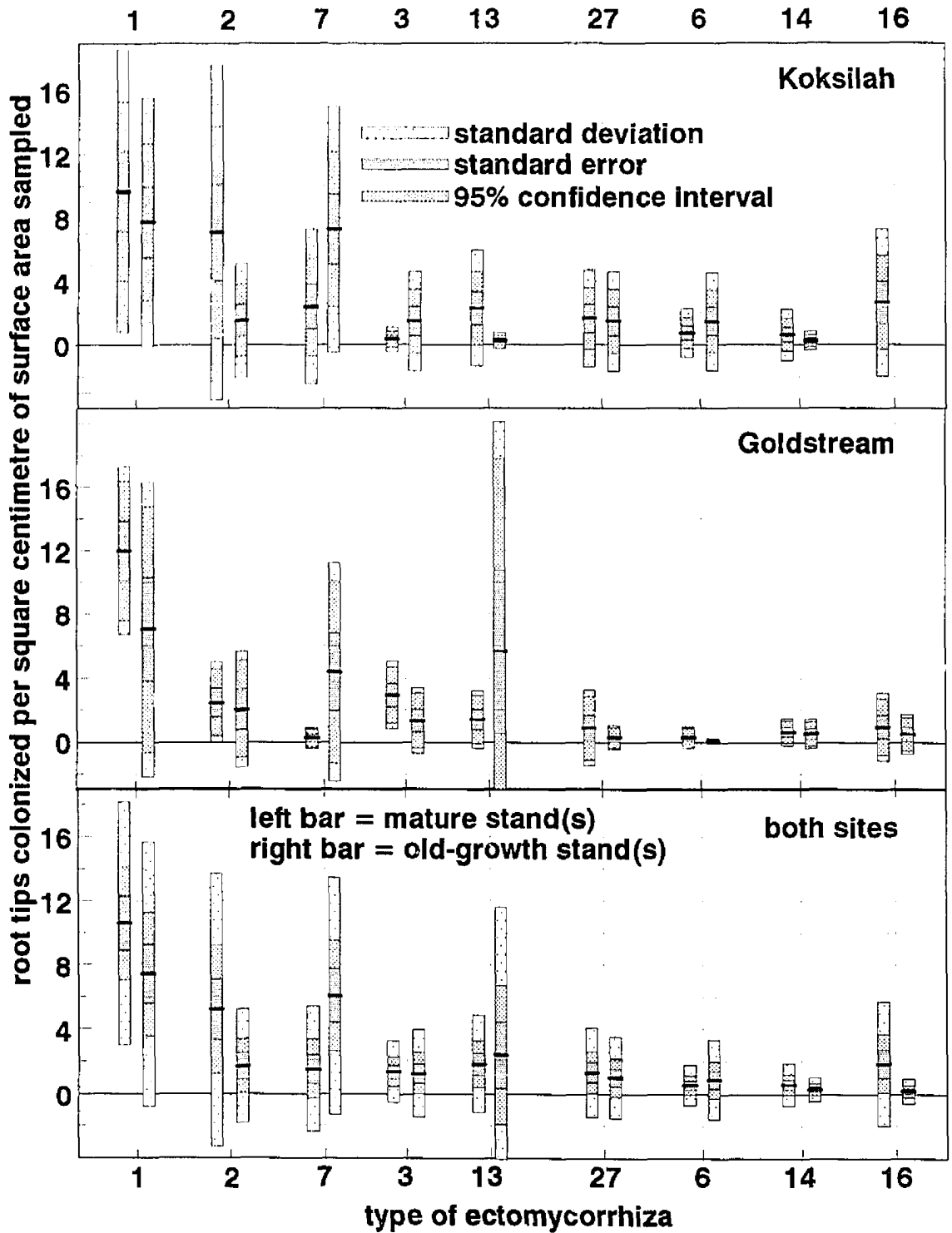
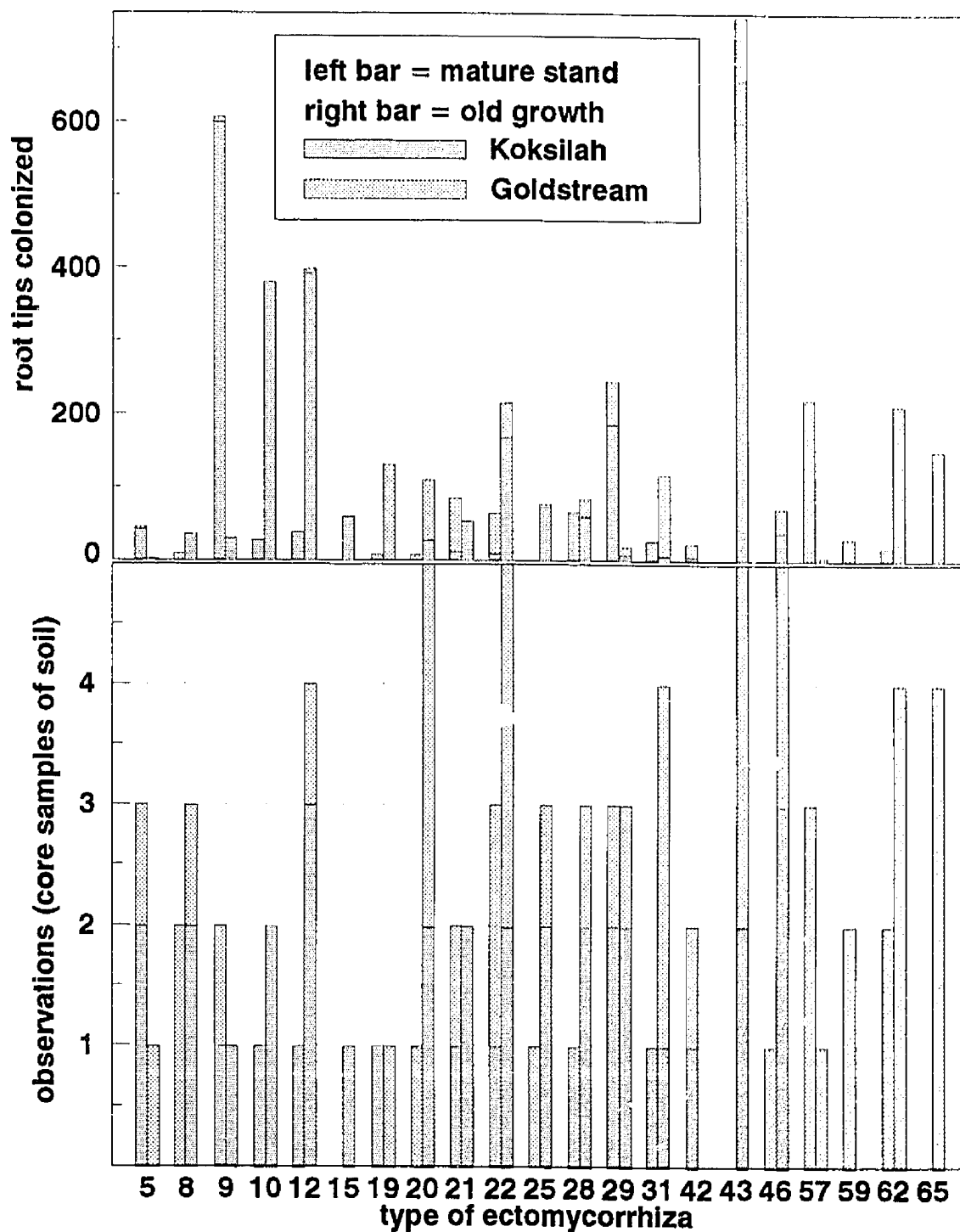


Figure 2.4. Abundance of the nine most frequent ectomycorrhizal types in old-growth and mature stands at two sites. 1 = *Cenococcum geophilum*, 2 = *Hysterangium*?, 7 = *Rhizopogon vinicolor*, 3 = *Piloderma*, 13 = *Lactarius deliciosus*



**Figure 2.5. Rooting density of the nine most frequent ectomycorrhizal types in old-growth and mature stands at two sites**



**Figure 2.6.** Abundance and frequency of less frequent types in mature (left bars) and old growth (right bars) at two sites. Only the more common or distinctive types (class=a) are included.

## Chapter Three -- Distribution of ectomycorrhizas related to the soil environment

### Introduction

Recent studies have shown that some ectomycorrhizas may function not only as extensions of root systems, absorbing available nutrients, but are probably responsible for the breakdown of complex organic substrates in the forest floor (Abuzinadah et al. 1986, Griffiths et al. 1990). Little is known of the roles of different ectomycorrhizal fungi in forest soils. The importance of ectomycorrhizal diversity is unclear, although Perry et al. (1987) consider it valuable for ecosystem stability. Some ectomycorrhizal fungi have been found more often in certain habitat types such as disturbed areas (Danielson and Pruden 1990), forest floor or mineral soil (Danielson and Visser 1989) and decayed stumps (Zak 1969).

Surveys of fungal sporophores in Europe showed that many species fruited only on specific soil types (Cooke 1948). Rarely have physiological or ecological specializations, or ecological tolerances, been demonstrated to explain patterns of distribution of ectomycorrhizae. An often-mentioned exception is the ability of *Cenococcum geophilum* to tolerate low water potentials in vitro (Mexal and Reid 1973, Pigott 1982, Theodorou 1978) and its association with dry habitats (Marks and Foster 1973, Pilz and Perry 1984, Worley and Hacskeylo 1959).

Harvey et al. (1978) found that during the dry summer months most ectomycorrhizae in a mature Douglas-fir/larch forest were located in decayed wood within the forest floor and consequently recommended that large woody debris not be removed from sites during harvesting. Improved understanding of the use of various soil environments by

ectomycorrhizal fungi to obtain nutrients could have other practical implications. Techniques for maintenance or enhancement of ectomycorrhizal diversity in forests could involve management of other tree species, understory species, or coarse woody debris (snags, stumps and logs). An objective of this study was to relate the distribution of ectomycorrhizas to types of substrate and levels of nutrients in the soil. In particular, I wanted to determine whether logs, stumps and soil near boles contained ectomycorrhizas different from those in soil elsewhere.

## Literature review -- Ectomycorrhizas and the cycling of nitrogen in temperate coniferous forests

**1. Introduction.** Within the wide variation of structure and nutrient regime of soils under temperate conifers, some generalizations are possible (Pritchett and Fisher 1987). Fertilization experiments show that nitrogen deficiency frequently limits growth, especially in the coldest climatic regions, where it is immobilized in the forest floor (=organic layer) and in the warmest regions if forests there are intensively managed over short rotations (Pritchett and Fisher 1987, Miller 1988). Nitrogen is the most limiting nutrient for Douglas-fir on southern Vancouver Island (Brix 1991). Unlike most agricultural soils, forests have a surface organic layer where most decomposition and uptake of nutrients occur. Decaying conifer litter produces organic acids that eluviate bases, creating an acidic soil environment. The underlying mineral soil layers usually contain a much lower concentration of nitrogen, which is moreover in less available forms such as humic compounds (Pritchett and Fisher 1987, Vitousek et al. 1982).

Weathering of mineral soil provides significant inputs of potassium and phosphorous, but almost no nitrogen (Pritchett and Fisher 1987). Nitrogen enters forests in precipitation and by biological fixation. The thickness and importance of the forest floor is greater in cooler soils or extremely wet or dry soils, which often have a sharp boundary between mineral soil and a dense, acidic "mor" humus. Soils that are warmer, richer, or of moderate moisture regime, are often characterized by a transition zone of "mull" humus between mineral soil and a thinner forest floor. Humus types form a continuum between these two extremes, along a gradient of density, acidity and mineral particle content (Bernier 1968). Intermediate types are called "moder" humus.

Because rates of colonization of absorbing root surfaces are very high (usually >90%) (Meyer 1973), ectomycorrhizas are responsible for nearly all the nitrogen (and other nutrients) that moves from soil to tree. Hence the importance of considering the role of ectomycorrhizas in the dynamics of nitrogen in forest soils.

**2. Nitrogen cycling.** Nitrogen enters forest ecosystems with (in order of magnitude) fixation of atmospheric N<sub>2</sub>, dust and aerosols, precipitation, weathering and fertilization (Pritchett and Fisher 1987). Symbiotic bacteria fix large amounts of nitrogen. *Frankia* fixes 100 Kg/ha/yr of nitrogen in stands of alder or snowbrush (*Ceanothus*) (Binkley et al. 1982, Pritchett and Fisher 1987). *Rhizobium* spp. with legumes and cyanobacteria in lichens fix roughly 10 Kg/ha/yr (Pritchett and Fisher 1987). One to two Kg/ha/yr N can be fixed by cyanobacteria on mosses, or by free-living bacteria in logs, in the rhizosphere, or on mycorrhizas (Fogel 1980, Li et al. 1990, Pritchett and Fisher 1987).

Application of 100-200 Kg N/ha of nitrogen fertilizer once per rotation is cost effective in many locations (Binkley 1986). The duration of response to nitrogen fertilizer is usually only 10-12 years (often only 5-6) and increases with tree age, suggesting that the applied nitrogen rapidly moves into the tree and into unavailable forms and that redistribution of nitrogen stored in the tree, including mycorrhizas, may account for the duration of the response (Miller 1988). Inputs from the atmosphere in dust, aerosols and precipitation are small (ca. 2-3 Kg/ha/yr) but significant over time. These inputs are several times greater near sources of air-pollution.

Major losses of nitrogen occur during and after burning or harvesting of forests and during or after site preparation. In contrast, losses from undisturbed forests due to leaching, runoff, or denitrification are negligible

compared to natural inputs (Pritchett and Fisher 1987). Nitrogen accumulates in the soil as forests age, although much of this is immobilized in organic matter (Heilman 1966, Miller 1988, Tamm et al. 1960, Turner 1977). Disturbance may be required to create soil conditions favourable for mineralization of organic nitrogen or for the establishment of N-fixing vegetation (Kimmins 1987). Depending on the amount of organic matter burnt, 20-200 Kg/ha of nitrogen is lost with volatile compounds during moderate fires, compared to 1000 Kg/ha in an intense fire (Binkley 1986, Pritchett and Fisher 1987). The amount of nitrogen lost during a fire is roughly proportional to the time between fires, with the result that the long-term losses average ca. 2-3 Kg/ha/yr. For example, prescribed burns performed once or twice during a 40 yr rotation of pine can remove 20 Kg/ha at 20 yr intervals. Higher pH of soil after a fire promotes N-fixation that replaces some of this loss (Kimmins 1987). Harvest losses usually amount to 2-10 Kg/ha per year of the rotation period (Pritchett and Fisher 1987), but can be several times greater if limbs, foliage, roots, or litter are removed in addition to the boles (Bengston 1981, Kimmins 1987, Pritchett and Fisher 1987). Leaching and runoff losses in undisturbed forests are low, e.g. 0.6 Kg/ha/yr (Gessel et al. 1973, Pritchett and Fisher 1987) and are 10-100 times less than the rates of mineralization and uptake from the soil (Vitousek et al. 1982). Leaching losses after disturbance are usually low, thanks to ammonia volatilization, fixation on clay and immobilization in organisms (Vitousek et al. 1982). When they do occur, leaching losses are an undesirable loss of nitrogen capital in the forest ecosystem and an undesirable input to aquatic ecosystems. From the latter standpoint, volatilization is preferable to leaching. Losses of nitrogen due to leaching are least on poor sites with organic matter of high C/N ratio (Vitousek et al. 1982, Edmonds 1987).

**3. The role of ectomycorrhizas.** Mycorrhizal fungi are a key link between roots and the soil animals, bacteria and saprotrophic fungi that break down organic detritus. Not only do ectomycorrhizas function in the decomposition of litter and uptake of nitrogen by trees, but they are themselves subject to decomposition. Below-ground cycling of nitrogen and organic matter between soil and roots may be as important as the larger cycle of uptake, primary production, litterfall and decomposition, but is not as well-studied. Roots and ectomycorrhizas in a Douglas-fir forest contained more nitrogen (and other nutrients except Ca) than other soil inhabitants and the forest floor (Fogel and Hunt 1983); and 43% of the nitrogen released in the forest was from ectomycorrhizas, the remainder accounted for primarily by soil organisms (Fogel 1980). Most absorbing roots of forest trees are mycorrhizal and most coniferous forest trees form ectomycorrhizas. Despite this, practical difficulties have prevented consideration of mycorrhizas in most studies of nutrient cycling (Fogel 1980).

Extramatrerial hyphae and rhizomorphs of ectomycorrhizal fungi can function as extensions of the root system by acquiring nitrogen in ammonium, nitrate, or amino-acid nitrogen and transferring it to the host (Trofymow and van den Driessche 1991). Based on their ability to decompose and use proteins *in vitro*, ectomycorrhizal fungi can be categorized as protein-fungi, non-protein fungi, or intermediate (Abuzinadah and Read 1986, Abuzinadah *et al.* 1986). This ability allows seedlings with some fungal partners to use proteins as their sole source of nitrogen (Abuzinadah and Read 1986a). The capability of some ectomycorrhizal fungi to degrade proteins is similar to that of saprotrophic, litter-decomposing fungi. The former, however, require a low concentration of a simple carbon or nitrogen compound to begin decomposing protein. Few ectomycorrhizal fungi of those tested were able to use humus

as a source of nitrogen (Lundeberg 1970). This helps explain why humus accumulates and why most ectomycorrhizas are in the F-layer rather than the H-layer. Proteins, peptides, amino-acids and amino-sugars are the predominant N-containing compounds in the litter and fragmented layers (Vitousek et al. 1982). Microbial tissue is likely the most important source of protein for ectomycorrhizas (Abuzinadah et al. 1986). These observations suggest that the nitrogen cycle is "tighter" than previously thought (e.g. Raven et al. 1978), allowing ectomycorrhizal fungi to compete with saprotrophs for organic sources of N and reducing the potential for loss of nitrogen by leaching (Abuzinadah et al. 1986). There is also some evidence that some ectomycorrhizal fungi can decompose lignin and cellulose (Cromack 1985, Harley and Smith 1983, Lundeberg 1970, Maijala et al. 1991).

The "Gadgil effect" refers to an apparent inhibition of decomposition of litter by ectomycorrhizal fungi (Gadgil and Gadgil 1975). When they excluded mycorrhizas, litter under radiata pine decomposed faster, perhaps because ectomycorrhizal fungi competed with or antagonized non-mycorrhizal decomposers of litter. Conversely, certain ectomycorrhizal fungi intensively colonize localized areas of forest floor, resulting in "mats" of soil, roots, rhizomorphs and mycelium, that have higher pH, higher levels of available nutrients, faster decomposition and nutrient turnover and greater biomass than other areas of the forest floor (Cromack 1990, Entry et al. 1991, Griffiths et al. 1990). Although decomposition is faster in mats than non-mat soil (which still contains ectomycorrhizas), the Gadgil effect could still be working in both mat and non-mat soil--without ectomycorrhizas, decomposition might be even faster.

The age of a stand of trees influences nitrogen dynamics and the role of ectomycorrhizas in nitrogen cycling. Even-aged stands develop following major disturbances such as

fire, clearcutting or windthrow. Changes in the soil environment following these events can increase levels of simpler forms of nitrogen in the soil and result in the movement of N into the mineral soil (Gessel et al. 1973, Kimmins 1987). Ectomycorrhizal fungi that predominate on seedlings and young trees ("early-stage" fungi) tend to be non-protein fungi, while those on older trees ("late-stage fungi") are mostly protein-fungi (Finlay and Frostegard 1990). Early-stage fungi may be better adapted to extract simple forms of nitrogen from mineral soil than to use more complex sources in organic matter. Nitrogen immobilization in organic matter as stands age (Heilman 1966, Miller 1988, Pritchett and Fisher 1987, Turner 1977, Vitousek et al. 1982) may result in a need for ectomycorrhizal fungi that can access complex N-containing compounds.

Ectomycorrhizas also play an important role in the cycling of other nutrients. Extramatrical hyphae can assimilate and transfer potassium and phosphorous to the host or to the mantle for storage (Beever and Burns 1980, Trofymow and van den Driessche 1991). Several fungi secrete oxalic acid that weathers P out of mineral soil (Cromack et al. 1979, Malajczuk and Cromack 1982). Some ectomycorrhizal fungi that form siderophores in culture may be able to increase the availability of Fe and other metals to trees (Szaniszlo et al. 1981).

## Methods and materials

The sites studied, methods of site selection and sampling design are described in Chapter 2. Methods of sampling, storage of soil, extraction of ectomycorrhizas, separation of types and description of types are described in Chapter 1.

**1. Nutrient analysis.** One half of each soil sample was dried and prepared for determination of pH, percent carbon, percent nitrogen, mineralizable nitrogen and extractable phosphorous. Samples of LFH materials were ground in a Wiley mill with a 1 mm sieve. Mineral soil was sieved to separate > 2 mm and < 2 mm fractions. For percent carbon and percent nitrogen determinations, part of the < 2 mm fraction of each sample was ground to a powder using a Sieb-Technic mill. Measurements of pH were made in a 1:4 (1:2 for mineral soil) ratio of soil to 0.01M CaCl<sub>2</sub> (Kalra and Maynard 1991). Percent carbon and nitrogen were determined by combustion-- %N using a modified Dumas procedure in a LECO 601-700 system (LECO 1985) and %C by the LECO CR-12 carbon system in a LECO 781-600 system (LECO 1981). Mineralizable nitrogen (mg/g) was determined by anaerobic incubation for two weeks (Bremner 1966). Extractable phosphorous was determined by a dilute acid-fluoride extract followed by spectrophotometric analysis (Kalra and Maynard 1991). Gravimetric moisture concentration was determined by weighing soil before and after drying.

**2. Data analysis.** Unless otherwise stated, analyses were performed with the aid of *Statistical Analysis Software (SAS) Inc.* procedures (SAS Institute 1985). To check for differences in soil habitats analyses of variance were run with PROC GLM, using the model "dependent variable = habitat age site habitat\*age habitat\*site age\*site habitat\*age\*site ". The assumption of homogeneity of variances was tested with scatter plots of residuals versus predicted values and

of standard deviations versus means for the values of the dependent variable. Normality of residuals was tested using PROC UNIVARIATE. Logarithmic, square-root or arcsine transforms were used if standard deviations varied by more than a factor of two or if skewness or kurtosis were greater than one in absolute value. Main effects or interactions were removed from the model if not significant at a confidence level of 20%. Due to uneven replication, adjusted means (least-squares means) were sometimes compared using t-tests. If transformations were not effective then a one-way non-parametric analysis of variance was performed using PROC NPAR1WAY followed by Dunn's multiple comparison procedure (Zar 1984). Non-parametric tests were run with age classes pooled as well as independently for each age class.

## Results

**1. Description of soil habitats.** The relative extents of five broad classes of soil-habitat at each site are listed in Table 3.1. Logs and stumps, LFH materials, and mineral soil differed in mean nutrient concentration and other parameters. Differences within these three groups were relatively minor. Results of statistical analyses are summarized in Table 3.2 and below.

**a. Logs and stumps.** Logs and stumps had a pH (3.3, 3.1 respectively) that was a full unit lower than in other habitats; and levels of extractable phosphorous (31, 20 ppm) which were 1/2-1/4 of levels in the other habitats. Less significantly, logs and stumps differed from other habitats in other parameters. Nitrogen concentration (0.45%) was 20-50% less than that of the forest floor elsewhere, but 3X greater than that of the mineral soil. Levels of mineralizable nitrogen (130, 120 ppm) in logs and stumps were similar to those of the forest floor near boies and elsewhere, but were only 1/3-1/4 of the level in the forest floor over rock and gravel. Mineralizable nitrogen levels were five times higher in logs and stumps than in mineral soil. Carbon concentration in logs and stumps (51%, 52% respectively) was 10-15% greater than that of the forest floor elsewhere. Although thicknesses of litter and fragmented litter on logs and stumps were only slightly lower than in the forest floor elsewhere, the humus layer was often absent and mean thicknesses of humus (5.1, 1.5 mm) were only 1/5 - 1/10 the values for the forest floor elsewhere. Moisture concentrations (67, 59%) were only slightly greater than those of the forest floor elsewhere and were 2-3X the level in mineral soil.

**b. Forest floor over mineral soil.** The pH of the forest floor over mineral soil (range 3.8-4.6) was 1/2 to a full point greater than in logs and stumps, but was similar to

that in mineral soil. Levels of extractable phosphorous and total and mineralizable nitrogen were 50-100% greater in the forest floor over mineral soil than in stumps and logs and 2-5X greater than in mineral soil. Humus layers were 5-10X thicker than those on logs and stumps and moisture concentration was 2-3X that of mineral soil.

The forest floor near boles contained much larger amounts of bark than elsewhere in the forest floor, resulting in thicker F layers and had a pH of 3.8, somewhat lower than elsewhere in the forest floor. Otherwise the forest floor at boles was similar to that elsewhere.

The forest floor over rock and gravel was richer in nutrients than in the "Bole" and "Other" habitats. Total nitrogen was 50% greater and mineralizable nitrogen was twice as high. The mean level of extractable phosphorous was 50-100% greater, but this was not statistically significant at 5%.

**c. Mineral soil.** Mineral soil had a pH (4.3, 4.7) similar to that of the forest floor, about one unit higher than in logs and stumps. Total nitrogen concentration of mineral soil (0.15%) was about 1/4 that of decayed wood and LFH, and mineralizable nitrogen was 1/5-1/10 as great as in decayed wood and LFH. Extractable phosphorous levels (56, 55 ppm) were similar to levels in LFH and logs and 2-3X the level in stumps. Carbon concentration (6.0, 5.3%) was 1/5 to 1/10 and moisture concentration was 1/3 levels in other habitats.

Within habitats, age classes were very similar in soil nutrient levels. Analysis of variance showed an effect of site (confounded with season) in many cases.

**2. Abundance and frequency of ectomycorrhizal types in soil-habitats.** Habitats differed in richness of types and abundance of ectomycorrhizal root tips (Table 3.3). Mineral soil, stumps and the forest floor over rock had lower densities of root tips and types than the forest floor

over mineral soil. Number of types and number of root tips were correlated with a coefficient of .59 ( $p=.0001$ ). Of the 18 most frequent types, 14 occurred in stumps or logs and 12 of those 14 were both more abundant and more frequent in logs than in stumps (Tables 3.3, 3.4). The forest floor near boles was similar to the forest floor elsewhere in abundance and frequency of each type and in abundance of all types (Tables 3.3, 3.4). The only possible exception was *Lactarius deliciosus*, which was not in any of 24 samples of LFH from near boles, but was in nine of 30 samples of LFH from the "other" habitat.

Five other types were significantly more abundant in some habitats than others (Table 3.3). *Cenococcum geophilum* was more abundant in LFH near bole than in LFH over rock and was more abundant in LFH than in mineral soil. Nevertheless, *Cenococcum geophilum* was abundant and frequent in all habitats. *Hysterangium* was more abundant in LFH over rock than in mineral soil. *Piloderma fallax* was most frequently found in logs and stumps, but was on average most abundant in LFH at boles. *Piloderma fallax* was often found in small pieces of wood or bark in samples of LFH, and overall was more abundant in decayed wood or bark than in other substrates. Type em12 was more abundant in LFH over rock than in mineral soil and stumps (only 5 occurrences total). In only a few cases did habitat effects depend on age class of the stand. In old growth only, *Lactarius deliciosus* was more abundant in the forest floor over mineral soil than in wood, the forest floor near boles, or the forest floor over rock ( $p=.03$ ), and type 12 was more abundant in the forest floor over rock than in all other habitats except the forest floor near boles. In mature stands only, type 14 was more abundant in wood than in other habitats.

Type 29, absent in LFH and wood, was unique among the types observed in its significant preference for mineral

soil (Tables 3.3-3.5). *Cenococcum geophilum*, *Rhizopogon vinicolor* and *Lactarius deliciosus* were abundant in both mineral soil and organic material. *Hysterangium*, *Piloderma fallax* and type 27 were almost exclusively found in organic material (LFH or wood).

**3. Relationship of ectomycorrhizal types and soil nutrients.** Table 3.6 lists correlations of soil nutrients with the abundance of each of the 18 most frequent types, the total number of ectomycorrhizal root tips per volume and the number of types per volume. Number of types and total abundance (root tips per litre) were correlated with total nitrogen ( $r=.24$  and  $.21$ ;  $p=.0004$  and  $.02$  respectively). Total ectomycorrhizal abundance was weakly correlated with carbon concentration ( $r=.17$ ,  $p=.04$ ). The most significant (largest probability values) correlations between densities of individual types and nutrients were *Cenococcum geophilum* with total nitrogen ( $r=.29$ ,  $p=.01$ ), type 8 with extractable phosphorous ( $r=.55$ ,  $p=.0001$ ) and mineralizable nitrogen ( $r=.25$ ,  $p=.002$ ), type 12 with extractable phosphorous ( $r=.37$ ,  $p=.0001$ ) and mineralizable nitrogen ( $r=.31$ ,  $p=.0002$ ), and type 16 with total nitrogen ( $r=.25$ ,  $p=.003$ ). Other correlations significant on a comparison-wise basis were *Hysterangium* with total nitrogen ( $r=.24$ ,  $p=.004$ ), *Piloderma fallax* with total carbon ( $r=.18$ ,  $p=.04$ ), type 6 with total nitrogen ( $r=.12$ ,  $p=.04$ ) and type 29 with total carbon and total nitrogen ( $r=-.21$ ,  $-.17$ ;  $p=.01$ ,  $.04$  respectively). Levels of soil nutrients were themselves positively correlated (Table 3.7).

Scatter plots of nutrient-levels versus abundances of types in root tips per litre showed that ectomycorrhizal root tips were abundant over a wide range of pH. Densities of tips of types 7, 12 and 46 were greatest at pH 3.5-4.7. Types 7, 27 and 46 were most abundant where total nitrogen was 0.3-1.1%. *Cenococcum geophilum* was abundant over a wide range of nitrogen and pH. Type 12 was most abundant at 600-

800 ppm mineralizable nitrogen. The number of types was greatest at pH 3.5-4.5 and total nitrogen of 0.4 to 1.1%.

**4. Correlation and co-occurrence of types.** The most obvious relationship between types was that of *Cenococcum geophilum* and *Piloderma fallax* (Tables 3.8, 3.9). Their densities were correlated ( $r=.5$ ,  $p=.0001$ ), which was apparent in a scatterplot, and contingency table analysis showed that their occurrences were not independent ( $p=.002$ ). *Rhizopogon vinicolor* and type 46 was the only other pair that showed both significant correlation and co-occurrence ( $r=.26$ ,  $p=.01$ ;  $p=.03$ ) (Tables 3.8, 3.9).

## Discussion

The broad classes of soil habitat differed in both their chemistry and their ectomycorrhizas. The forest floor over bedrock or gravel contained more nitrogen and extractable phosphorous and more ectomycorrhizas of *Hysterangium*, type 8 and type 12, than the other habitats. The occurrence of these fungi there was perhaps related to the high nutrient levels. Roots and fungi translocate nutrients from the forest floor to ectomycorrhizas in the mineral soil. Leaching and the movements of soil animals also move nutrients from the forest floor into the mineral soil. There is probably less movement of nutrients out of the forest floor where it overlies bedrock or gravel, perhaps explaining the higher nutrient levels there. The higher nutrient levels in LFH over rock compared to LFH over mineral soil may also be contributed to by the activity of *Hysterangium* species, which are known to form dense mats of mycelium and soil that are higher in nutrients than non-mat soil (Griffiths et al. 1991). Another reason for elevated nutrient levels in the forest floor over bedrock may be increased turnover of nutrients in soil biota, due to periodic drying and saturation. The significant correlations of the abundance of types 8 and 12 with mineralizable nitrogen and extractable phosphorous levels may indicate habitat preferences. Alternatively, it may be that the nutrient concentration of the soil sample was the result of the abundance of ectomycorrhizal tips rather than *vice-versa* (depending on the relative nutrient concentration of ectomycorrhizas and the surrounding soil).

Levels of extractable phosphorous may have increased during storage of moist soil, especially in LFH materials. This bias may have exaggerated or biased apparent relationships, although methods of storage and drying were similar for all samples. Many samples of a thin layer of

forest floor over rock were without roots, perhaps because of frequent drying. Considering only those samples with ectomycorrhizas, the forest floor over rock had a richness and density of ectomycorrhizas similar to the forest floor over mineral soil.

The forest floor near boles had more decaying bark, more fragmented litter and less ectomycorrhizas of *Lactarius deliciosus* than other habitats. The absence of *L. deliciosus* from all samples taken near boles and from the forest floor over rock is unexplained and was perhaps due to chance. The abundance of ectomycorrhizas of *Piloderma fallax* near boles is probably related to the higher carbon concentration and lower pH of the forest floor near boles compared to the forest floor elsewhere, likely caused by the content of decaying bark.

That *Piloderma fallax* was associated with woody debris is in accord with previous reports (e.g. Agerer 1993). Type 14 was also more common in wood than other habitats. The lower density of ectomycorrhizal rooting in stumps than logs may be due to lower moisture concentration and thinner and less frequent layers of LFH materials. There is little evidence to explain the apparent preference of some ectomycorrhizal fungi for woody substrates. Are these fungi more adept at capturing or degrading nitrogen sources typical of logs and stumps? Perhaps ectomycorrhizal fungi (e.g. *Byssoporia terrestris*) that are closely related to wood-rooting saprotrophs have retained adaptations for acquiring nitrogen in woody debris.

Distinct patterns of ectomycorrhizal occurrence in mineral soil compared to decayed wood and the forest floor were not surprising considering the latter habitats contained 5-10X more carbon, nitrogen and mineralizable nitrogen. The restriction of the bright greenish-yellow type 29 to mineral soil was the most dramatic relationship observed between types and habitats, indicating that the

mycobiont of type 29 may have unusual abilities to mineralize or extract nutrients from mineral soil. *Hysterangium*, *Piloderma fallax* and type 27 were absent or nearly so from mineral soil, likely the result of dependency on organic substrates.

*Cenococcum geophilum*, *Rhizopogon vinicolor* and *Lactarius deliciosus* were both common and abundant in mineral soil, the forest floor, logs and stumps, probably indicating wide environmental tolerances and a lack of specificity for substrate. Both *C. geophilum* and *R. vinicolor* are known for tolerance of dry conditions (Marks and Foster, 1973; Pilz and Perry, 1984; Worley and Hacskeylo, 1959), which may account for their abundance. Both were however positively correlated with the moisture concentration of the soil samples.

That there were few strong correlations between the abundance of types and nutrient levels may have been due to the presence of micro-habitats with different nutrient concentrations within a soil core, or due to a limited range of nutrient levels at the sites, or due other aspects of the soil environment more influential than the parameters measured. More ectomycorrhizal tips were present in the forest floor than in mineral soil or decayed wood, but other than this general observation, abundance of ectomycorrhizas was only weakly correlated with nutrient levels, or pH. Erland (1992) found in pot culture that most ectomycorrhizal fungi were tolerant of soils with pH in the range 3.5 to 7 (lower values not studied) and that pH effects were most often the result of the metal content of the soil. Except for stumps and logs, the soils studied at Koksilah and Goldstream were within this range and no correlation was found between the occurrence of ectomycorrhizas and pH.

There are other probable influences on the distribution of ectomycorrhizas. Ectomycorrhizal fungi modify their environment. Mat-forming fungi are a prominent example

(Cromack et al. 1979). Perhaps fungi that accumulate high concentrations of metals, e.g. *Russula ochroleuca* (Lesova et al.) create modified substrates that they are better able to colonize. Deacon and Fleming (1992) have hypothesized that most late-stage fungi are stress tolerant. Potential environmental stresses may include a variety of chemicals and organisms. Perhaps it is not surprising that types occurred in samples with a wide range of pH and nutrient levels when many other soil variables are in play. Although the differences between habitats or ectomycorrhizas mentioned above are statistically significant with probability values less than .05 and mostly less than .01, some are based on comparison-wise testing and therefore there is a greater chance that one or more of the differences noted is spurious.

Interpretation of the data requires an awareness of the limited scope of the study. Each observation was of ectomycorrhizal root tips only and does not precisely indicate the location of rhizomorphs. There may be important differences between the micro-habitats occupied by the ectomycorrhizal root tips and the micro-habitats occupied by the attached rhizomorphs. For instance, tips found in mineral soil may have rhizomorphs in the overlaying forest floor or vice-versa. Furthermore, each broad class of habitat that was examined had a diversity of micro-habitats. More ectomycorrhizal tips and more types were found in the near-bole habitat than elsewhere, perhaps because of the presence of both small woody debris and other litter types there.

Similarly, samples from logs and stumps were analyzed as a unit including any surface layers of LFH materials. The only routine separation of materials within a soil sample was to separate the mineral soil from the overlying organic materials. I suspect that some types occur predominantly in particular types of litter or humus. Litter contained

leaves of various kinds, twigs, cones, small pieces of decaying wood and the corpses and faeces of microfauna. It is impractical to survey all types in all micro-habitats and collect enough data to gain much information. It would be more fruitful to survey only certain micro-habitats or certain types, preferably those without extensive systems of rhizomorphs.

Ectomycorrhizal systems tend to be distributed in a spatially clumped distribution (Dahlberg and Stenlid 1990) and systems vary widely in number of tips. Therefore a large sample size is required to obtain precise means. An example of this problem is in comparing ectomycorrhizal abundance for logs and stumps. Most types were more abundant in logs than stumps, but over 600 tips of one type were found in one sample from a stump, so that overall the mean number of ectomycorrhizal tips per volume in stumps was twice that in logs. It was found that one half of a 5 cm diameter soil sample contained almost exactly the same number of types on average as a full sample. A drawback of smaller core samples, however, is that fewer tips are available for identification of the types. As well as a limited spatial coverage, this survey considered only two times within the year--May and November. It is possible that some of the patterns observed would change under drier or colder conditions. For instance, woody debris has been found to contain more active ectomycorrhizas than the forest floor during dry seasons (Amaranthus and Perry 1990, Harvey et al. 1978). Finally, the data are for only two sites and four 60 x 60 m plots. This study may have revealed only a small proportion of the community of ectomycorrhizas in a Douglas-fir forest.

Understanding the ecological role of ectomycorrhizas in relation to substrate types and other soil organisms is needed to evaluate their individual roles in the growth of forest trees. What would the impact be of the disappearance

of one or more ectomycorrhizal fungi from a stand? In agricultural systems the relative benefit of different arbuscular mycorrhizal fungi has been evaluated using pot culture, showing that fertilization can select for less beneficial symbionts (Johnson 1993). This approach is probably not suitable for ectomycorrhizal fungi, as their behaviours with tree seedlings in experimental conditions are unlikely to predict their behaviours in forests. Continued observation of ectomycorrhizas and ectomycorrhizal fungi in the field is necessary before the impacts of forest practices on ectomycorrhizas can be predicted or managed.

**Tables**

Table 3.1. Extents of five broad classes of soil-habitat (percent of plot area)					
Site and age class	Logs	Stumps	Other LFH	LFH over rock <sup>a</sup>	LFH near boles <sup>b</sup>
Koksilah each age class <sup>c</sup>	3.6	0.4	82	13	1.2
Goldstream mature	≈ 7-10	not measured	≈ 87	≈ 3	≈ 0.4
Goldstream old growth	≈ 7-10	not measured	≈ 90	≈ 0	≈ 1.0

a -- areas of LFH over bedrock or gravel, with no trees and few shrubs

b -- LFH and mineral soil within 5 (15) cm of dominant or codominant trees in mature (old-growth) stands

c -- amounts were approximately the same in each age class

Habitat	Logs	Stumps	Other LFH	LFH over rock <sup>a</sup>	LFH near boles <sup>b</sup>	Other mineral soil	Mineral soil near boles
pH	3.3 D <sup>c</sup>	3.1 D	4.5 AB	4.6 AB	3.8 C	4.7 A	4.3 B
Carbon (%)	51 AB	52 A	36 C	38 BC	42 ABC	6.0 D	5.3 D
extractable Phosphorous (µg/g)	31 CD	20 D	73 AB	110 A	53 ABC	73 ABC	55 ABC
Nitrogen (%)	0.45 C	0.44 C	0.76 B	1.05 A	0.71 B	0.15 D	0.15 D
Mineralizable Nitrogen (µg/g)	130 B	120 B	180 B	420 A	190 B	21 C	18 C
moisture (%)	67 A	59 AB	53 B	55 B	53 B	19 C	17 C
depth of core samples (mm)	135 AB	143 A	115 B	64 C	125 AB	---	---
thickness of litter in mature stands (mm)	14.7 A	8.7 A	13.5 A	12.3 A	12.9 A	---	---
thickness of litter in old growth (mm)	8.0 B	10.4 A	15 AB	13.1 AB	19 A	---	---
thickness of fragmented litter (mm)	12.3 B	10 B	16.8 AB	10 B	23 A	---	---
thickness of humus (mm)	5.1 B	1.5 B	19.1 A	15 A	28 A	---	---

a -- areas of LFH over bedrock or gravel, with no trees and few shrubs  
 b -- LFH and mineral soil within 5 (15) cm of dominant or codominant trees in mature (old-growth) stands

c -- means followed by the same letter are not significantly different at a confidence level of 5% according to a Tukey procedure, means separation was based on transformed data in some cases

Table 3.3. Abundance (root tips per litre) of the most frequent types in seven classes of soil-habitat (mean from three core samples (standard deviation))								
type	Habitat							p-val <sup>a</sup>
	Logs (n=26)	Stumps (n=23)	Other LFH (n=30)	LFH over rock (n=12)	LFH near boles (n=24)	Other mineral soil (n=26)	Mineral soil near boles (n=16)	
em1 <i>Cenococcum geophilum</i>	16.6 (21) AB <sup>b</sup>	14.3 (39) ABC	28.0 (40.5) AB	13.1 (24) BC	26.0 (39) A	2.4 (7.5) C	3.4 (7.6) C	0.0001 <sup>c</sup>
em2 <i>Hysterangium</i>	2.59 (4.23) AB	0.73 (2.9) AB	17.3 (47) A	60.4 (195) AB	15.1 (32) A	0.16 (0.84) B	0.42 (1.70) AB	0.001 <sup>c</sup>
em3 <i>Piloderma fallax</i>	3.3 (7.0)	1.90 (4.1)	2.9 (11.8)	0 (0)	5.5 (18.7)	0 (0)	0 (0)	0.01
em6	0.73 (2.3)	0.13 (0.64)	0.65 (2.6)	0 (0)	3.5 (16)	0.24 (1.24)	0 (0)	0.55
em7 <i>Rhizopogon vinicolor</i>	1.74 (5.2)	1.37 (4.5)	14.7 (43)	13.6 (47)	12.2 (22)	0.53 (2.1)	4.2 (11.5)	0.12 <sup>c</sup>
em8	0.14 (0.51)	0 (0)	0.32 (1.56)	1.95 (6.8)	0 (0)	0 (0)	0 (0)	0.33
em12	0.50 (2.5)	0 (0)	0 (0)	5.7 (13.4)	0.11 (0.53)	0 (0)	0 (0)	0.05
em13 <i>Lactarius deliciosus</i>	1.74 (5.2)	0.37 (1.77)	16.9 (60.5)	0 (0)	0 (0)	3.6 (9.0)	1.64 (4.5)	0.01
em14	1.75 (4.3)	0.52 (1.80)	0.48 (1.81)	0 (0)	0 (0)	1.57 (8.0)	0 (0)	0.08
em16	1.56 (8.0)	0 (0)	6.3 (21.4)	0 (0)	2.6 (8.5)	0.017 (0.087)	0 (0)	0.19
em20	1.11 (5.1)	0 (0)	0.030 (.17)	0.93 (3.2)	0.14 (0.69)	0.35 (1.78)	0 (0)	0.76

Table 3.3 (continued). Abundance (root tips per litre) of the most frequent types in seven classes of soil-habitat (mean from three core samples (standard deviation))								
type	Habitat							p-val <sup>a</sup>
	Logs (n=26)	Stumps (n=23)	Other LFH (n=30)	LFH over rock (n=12)	LFH near boles (n=24)	Other mineral soil (n=26)	Mineral soil near boles (n=16)	
em22	0 (0)	0 (0)	4.7 (16.3)	0 (0)	1.90 (5.2)	0.53 (1.95)	.071 (0.28)	0.21
em27	4.9 (13.4)	0.044 (0.21)	4.9 (22)	1.24 (4.3)	1.44 (5.1)	0 (0)	0 (0)	0.26
em29	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2.07 (6.2)	0.41 (1.17)	<b>0.03</b>
em31	0 (0)	0 (0)	1.47 (6.6)	0.23 (0.80)	4.33 (15.4)	0 (0)	0 (0)	0.30
em43	0 (0)	10.0 (43)	1.68 (7.8)	0 (0)	0 (0)	0 (0)	0 (0)	0.12
em46	0.32 (1.55)	0.13 (0.64)	0.34 (1.87)	0 (0)	0.94 (3.8)	0 (0)	0 (0)	0.32
em62	0 (0)	0 (0)	4.33 (22)	0 (0)	0.84 (3.6)	0.173 (0.89)	1.11 (4.5)	0.59
all types	46 (47) A	82 (27) A	114 (130) A	65 (77) A	129 (130) A	28 (49) A	24 (43) A	0.08 <sup>c</sup>
total tips/ total volume	47	33	109	78	101	20	22	
types per litre	1.22 (1.19) A	0.86 (1.41) A	3.58 (4.14) A	1.77 (1.73) A	3.52 (2.87) A	1.24 (1.80) A	1.24 (1.39) A	0.06 <sup>c</sup>

a -- probability value from a Kruskal-Wallis non-parametric analysis of variance, except where indicated by c; means separation by Dunn's procedure following non-parametric mean separation failed to reveal significant pairwise comparisons at a confidence level of 95%

b -- means followed by the same letter do not differ significantly at a confidence level of 95% according to a Tukey procedure, means separation was based on a logarithmic or square-root transformation

c -- probability value of main effect of habitat from F-test of analysis of variance

-- probability values  $\leq 5\%$  are in bold

Table 3.4. Frequency of the most frequent types in seven classes of soil-habitat (number of soil-samples <sup>a</sup> (percentage of samples containing ectomycorrhizas)								
type	Habitat							p-val <sup>b</sup>
	Logs (n=22)	Stumps (n=16)	Other LFH (n=21)	LFH over rock (n=7)	LFH near boles (n=22)	Other mineral soil (n=15)	Mineral soil near boles (n=10)	
em1 <i>Cenococcum geophilum</i>	21(95)	13(81)	19(90)	3(43)	18(82)	4(27)	4(40)	0.0001
em2 <i>Hysterangium</i>	10(45)	2(12)	12(57)	3(43)	10(45)	1(7)	1(10)	0.001
em3 <i>Piloderma fallax</i>	9(41)	5(31)	3(14)	0(0)	4(18)	0(0)	0(0)	
em6	4(18)	1(6)	2(10)	0(0)	3(14)	1(7)	0(0)	
em7 <i>Rhizopogon vinicolor</i>	6(27)	2(12)	7(33)	1(14)	8(36)	2(13)	2(20)	
em8	2(9)	0(0)	2(10)	1(14)	0(0)	0(0)	0(0)	
em12	2(9)	0(0)	0(0)	2(28)	1(5)	0(0)	0(0)	
em13 <i>Lactarius deliciosus</i>	4(18)	1(6)	9(43)	0(0)	0(0)	5(33)	2(20)	
em14	5(23)	2(12)	2(10)	0(0)	0(0)	1(7)	0(0)	
em16	1(5)	0(0)	4(19)	0(0)	3(14)	1(7)	0(0)	
em20	2(9)	0(0)	1(5)	1(14)	1(5)	1(7)	0(0)	
em22	0(0)	0(0)	4(19)	0(0)	3(14)	2(13)	1(10)	
em27	5(23)	1(4)	4(19)	1(14)	3(14)	0(0)	0(0)	
em29	0(0)	0(0)	0(0)	0(0)	0(0)	3(20)	3(20)	
em31	0(0)	0(0)	2(10)	1(14)	2(9)	0(0)	0(0)	
em43	0(0)	2(12)	3(14)	0(0)	0(0)	0(0)	0(0)	
em46	1(5)	1(6)	1(5)	0(0)	3(14)	0(0)	0(0)	
em62	1(5)	0(0)	2(10)	0(0)	2(9)	1(6)	1(10)	

a -- only those samples containing ectomycorrhizas were included in the analysis

b -- data was only sufficient to calculate chi-squared probability values for the two most common types

Table 3.5. Frequency of the most frequent types in the forest floor and mineral-soil (number of soil-samples (percentage of samples containing ectomycorrhizas)			
type	forest floor (n=88)	mineral soil (n=29)	Fisher's exact probability-value
em1 -- <i>Cenococcum geophilum</i>	74 (85)	10 (34)	<b>0.000001</b>
em2 -- <i>Hysterangium</i>	37 (42)	2 ( 7)	<b>0.0005</b>
em3 -- <i>Piloderma fallax</i>	21 (24)	0 ( 0)	<b>0.002</b>
em6	10 (11)	1 ( 3)	0.29
em7 -- <i>Rhizopogon vinicolor</i>	24 (27)	4 (14)	0.20
em8	5 ( 6)	0 ( 0)	0.33
em12	5 ( 6)	1 ( 3)	1.00
em13 -- <i>Lactarius deliciosus</i>	14 (16)	8 (28)	0.18
em14	9 (10)	1 ( 3)	0.45
em16	8 ( 9)	1 (3)	0.45
em20	5 ( 6)	1 ( 3)	1.00
em22	7 ( 8)	3 (10)	0.71
em27	14 (16)	0 ( 0)	<b>0.02</b>
em29	0 ( 0)	6 (21)	<b>0.0001</b>
em31	5 ( 6)	0 ( 0)	0.33
em43	5 ( 6)	0 ( 0)	0.33
em46	6 ( 7)	0 ( 0)	0.33
em62	5 ( 6)	2 ( 7)	1.00

probability values  $\leq 5\%$  are in bold

Table 3.6. Correlation of abundance of ectomycorrhizal types (root tips per litre) with nutrient levels in the forest floor and mineral soil of each sample (pearson coefficient, probability value <sup>a</sup> )							
	pH (n=73)	total carbon (n=93)	extractable phosphorous (n=102)	total nitrogen (n=99)	mineral- izable nitrogen (n=102)	moisture (%) (n=150)	
em1 -- <i>Cenococcum geophilum</i>	-0.05 0.62	<b>0.26 0.003</b>	-0.00 0.96	<b>0.29 0.01</b>	-0.09 0.26	<b>0.21 0.01</b>	
em2 -- <i>Hysterangium</i>	0.12 0.20	0.08 0.34	-0.02 0.83	0.24 <b>0.004</b>	0.14 0.08	-0.02 0.81	
em3 -- <i>Piloderma fallax</i>	-0.13 0.19	0.18 <b>0.04</b>	-0.00 0.98	-0.02 0.77	-0.04 0.58	0.10 0.20	
em6	-0.05 0.58	0.08 0.35	-0.05 0.55	0.12 <b>0.04</b>	0.06 0.45	0.09 0.25	
em7 -- <i>Rhizopogon vinicolor</i>	0.03 0.74	0.08 0.33	-0.01 0.90	0.14 0.10	0.05 0.50	0.09 0.28	
em8	0.10 0.32	0.02 0.84	<b>0.55 0.0001</b>	0.10 0.24	<b>0.25 0.002</b>	0.07 0.42	
em12	0.10 0.30	0.02 0.85	<b>0.37 0.0001</b>	0.14 0.10	<b>0.31 0.0002</b>	0.06 0.43	
em13 -- <i>Lactarius deliciosus</i>	0.15 0.12	0.00 1.00	0.05 0.54	0.11 0.19	-0.07 0.37	0.01 0.94	
em14	-0.14 0.13	0.04 0.68	-0.07 0.40	-0.09 0.30	-0.04 0.62	0.06 0.45	
em16	0.06 0.50	0.09 0.30	0.09 0.28	<b>0.25 0.003</b>	0.03 0.75	0.06 0.48	
em20	-0.01 0.91	0.05 0.53	-0.04 0.58	0.02 0.76	0.02 0.81	0.01 0.88	
em22	0.13 0.17	-0.05 0.58	0.02 0.83	0.09 0.29	-0.02 0.85	0.09 0.29	
em27	0.03 0.75	0.08 0.33	-0.04 0.60	0.14 0.09	-0.03 0.67	0.15 0.07	
em29	0.16 0.10	-0.21 <b>0.01</b>	-0.04 0.66	-0.17 <b>0.04</b>	-0.10 0.22	0.20 <b>0.01</b>	
em31	-0.03 0.74	0.09 0.28	-0.00 0.95	0.15 0.07	0.13 0.09	0.07 0.40	
em43	-0.00 0.99	0.09 0.30	0.03 0.70	0.02 0.82	-0.02 0.83	0.08 0.32	
em46	-0.02 0.83	0.04 0.63	-0.00 0.98	0.00 0.96	-0.05 0.52	0.07 0.42	
em62	-0.03 0.78	0.02 0.80	0.05 0.56	0.07 0.38	-0.06 0.44	0.01 0.86	
all types combined	-0.02 0.83	0.17 <b>0.04</b>	-0.01 0.87	0.21 <b>0.02</b>	0.07 0.41	0.23 <b>0.01</b>	
types per litre	0.02 0.30	0.10 0.24	0.09 0.29	0.24 <b>0.0004</b>	-0.07 0.37	0.21 <b>0.01</b>	

a -- coefficients  $\geq 25\%$  and probability values under 0.05 are in bold

	percent carbon	extractable phosphorous	percent nitrogen	mineralizable nitrogen	moisture (%)
pH	<b>-0.78<sup>a</sup></b> 0.0001 110	<b>0.47</b> 0.0001 111	0.03 0.71 111	0.09 0.34 111	<b>-0.60</b> 0.00 1
percent carbon		<b>-0.25</b> 0.004 134	<b>0.45</b> 0.0001 134	0.22 0.01 134	<b>0.82</b> 0.00
extractable phosphorous			0.16 0.06 140	<b>0.33</b> 0.0001 149	-0.09 0.27
percent nitrogen				<b>0.56</b> 0.0001 138	<b>0.45</b> 0.00
mineralizable nitrogen					<b>0.32</b> 0.00

a -- correlation coefficients  $\geq 25\%$  are in bold

pair of types	correlation coefficient	probability value
em1 -- <i>Cenococcum geophilum</i> , em3 -- <i>Piloderma fallax</i>	.50	.0001
em1 -- <i>Cenococcum geophilum</i> , em16	.28	.003
em7 -- <i>Rhizopogon vinicolor</i> , em12	.37	.0001
em7 -- <i>Rhizopogon vinicolor</i> , em27	.48	.0001
em7 -- <i>Rhizopogon vinicolor</i> , em46	.26	.006
em8, em12	.54	.0001
em8, em22	.25	.01
em13 -- <i>Lactarius deliciosus</i> , em62 -- <i>Russula</i>	.95	.0001

Table 3.9. Significant co-occurrences of ectomycorrhizal types (contingency table analysis)						
type A	type B	soil- samples with neither type	soil- samples with A but not B	soil samples with B but not A	soil- samples with both types	Fisher's exact probab- ility value
em1 -- <i>Cenococcum geophilum</i>	em2 -- <i>Hysterangium</i>	29	49	4	35	.002
em1 -- <i>Cenococcum geophilum</i>	em3 -- <i>Piloderma fallax</i>	32	64	1	20	.007
em1 -- <i>Cenococcum geophilum</i>	em12	28	83	5	1	.007
em1 -- <i>Cenococcum geophilum</i>	em27	33	70	0	14	.01
em2 -- <i>Hysterangium</i>	em31	77	35	1	4	.04
em7 -- <i>Rhizopogon vinicolor</i>	em46	87	24	2	4	.03
em13 -- <i>Lactarius deliciosus</i>	em14	90	17	5	5	.02

## General discussion

It is perhaps not surprising that the ectomycorrhizal communities of old-growth and mature stands were so similar, considering their proximity and the similarity of their vegetation and soil. The same fires that initiated the mature stands probably burnt in the old-growth stands, affecting the soil in a similar manner. It appears that many ectomycorrhizal fungi do not survive clearcutting (Amaranthus and Perry 1990, Borchers and Perry 1990, Perry *et al.* 1987) and therefore must depend on dispersal from older stands in order to colonize younger stands. Proximity of old-growth to maturing stands may be especially important for hypogeously fruiting species, which are dispersed mainly by small mammals. The proximity of old-growth stands and veteran trees that survived or escaped the fire that led to the start of the mature plots may have contributed to the resilience of the ectomycorrhizal community in the mature plots. The ectomycorrhizal community of mature plots in this study may also have been enriched by the presence of some old-growth legacy of large stumps and logs, although these were far fewer than in the old-growth plots.

Ectomycorrhizal succession probably progresses slowly if at all after 90 years of stand age in stands similar to those of this study. Differences in the ectomycorrhizas of mature and old-growth forests are more likely to occur where frequent or severe disturbance, lack of old-growth legacies, or lack of old-growth stands from which fungi can disperse has delayed or redirected ectomycorrhizal succession.

The Koksilah and Goldstream sites, being of poor to medium quality, probably have soil of lower nutrient levels

than good sites, resulting in an earlier transfer of nutrients from the mineral soil to more complex forms in the forest floor. Since it is hypothesized that this transfer may be a major cause of succession of ectomycorrhizas, succession may be delayed on good sites, leading to greater differences in the ectomycorrhizal communities of old-growth and 90-year-old stands. Moreover, stands on good sites generally have lower rotation ages, increasing the chance that ectomycorrhizal diversity is reduced where old growth is eliminated. Even if 80-year-old stands are able to support the diversity of ectomycorrhizal fungi found in old growth, the area of stands of that age in a commercial forest with an 80 year rotation may be insufficient for long-term maintenance of the fungi. It may be necessary to study the ectomycorrhizas of younger stands to determine if diversity of ectomycorrhizas can be maintained in commercial forests.

Despite the results of this study, Meyer's (1973) suggestion that the mycorrhizal community of old growth is probably not significantly different from that of mature forests seems premature. Enough data were collected to make conclusions about the most common ectomycorrhizas only. It is possible that less common species of ectomycorrhizal fungi may include some with niches peculiar to old-growth forests. Before much more is known of the diversity and habit of ectomycorrhizas in old-growth and mature Douglas-fir forests, one cannot dismiss the significance of ectomycorrhizal succession in mature stands. Recognizing the importance of biological diversity, including rare species, even small successional changes could be important.

It remains to be seen whether the trends of this study are true of other stands of Douglas-fir or of other forest

types. More research is needed before generalizations can be made about ectomycorrhizal succession beyond stand ages of 80 years. The distribution of the rarer ectomycorrhizas can only be ascertained by more intensive surveys of ectomycorrhizas. Surveys of sporocarps could provide valuable complementary information. It will also be necessary to examine stands subjected to different forestry practices at a number of sites. Future comparisons of old-growth and mature plots might best focus on the large stumps and logs that are absent from mature stands. There is still reason for concern that if refugia of old growth are not maintained throughout the landscape and instead large areas of forest are kept at ages less than 100 years, then soil conditions may gradually change, with losses of richness and diversity of ectomycorrhizas.

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**Appendix 1A. Ectomycorrhizal types -- contents of  
electronic database of characters for 69 types of  
ectomycorrhizas**

**1. Explanation of fields**

File : EM.DBF  
 Number of data records: 70  
 Date of last update : 11/22/94

Field	Field Name	Type	Width	Decimals
1	EM	Numeric	3	
2	GENUS	Character	20	
3	SPECIES	Character	20	
4	SN	Character	3	
5	DATE	Date	8	
6	NTIPS	Numeric	4	
7	TTOTAL	Numeric	5	
8	CTOTAL	Numeric	3	
9	CLASS	Character	1	
10	RAM	Numeric	1	
11	SYLEN	Numeric	2	
12	TIPDIAM	Numeric	4	
13	AXDIAM	Numeric	4	
14	TIPSHAPE	Character	1	
15	MORPHNOTE	Character	110	
16	SEEMANTLE	Logical	1	
17	SEEROOT	Logical	1	
18	MANTSURF	Numeric	2	
19	COLOUR	Character	5	
20	NEWCOLOUR	Character	5	
21	OLDCOLOUR	Character	5	
22	COLOURNOTE	Character	80	
23	RZFREQ	Character	1	

24	RZOCCUR	Character	50	
25	RZCONNECT	Numeric	2	
26	RZCOLOUR	Character	5	
27	RZDIAM	Numeric	3	
28	RZRAM	Character	50	
29	RZXS	Character	1	
30	RZSURF	Character	2	
31	RZNOTE	Character	80	
32	EH	Logical	1	
33	EHDENSITY	Character	1	
34	EHOCCUR	Character	50	
35	EHLPSHAPE	Character	40	
36	EHMORPNOTE	Character	70	
37	ETOH	Character	50	
38	FESO4	Character	50	
39	KOH	Character	50	
40	LACTICACID	Character	50	
41	MEJTZERS	Character	50	
42	SULFOVAN	Character	50	
43	AF254	Character	5	
44	AF366	Character	5	
45	LACT	Logical	1	
46	LACTDIST	Character	50	
47	LACTDIAM	Numeric	4	1
48	LACTRAM	Character	30	
49	LACTCONT	Character	30	
50	LACTCOLOUR	Character	5	
51	LACTSEPTA	Character	30	
52	OMANTYPE	Numeric	1	
53	OMCELLSHAP	Character	50	
54	OMSEPTAVIS	Logical	1	
55	OMCLAMPS	Logical	1	
56	OMSEPTANOT	Character	50	

57	OMCELLWIDE	Numeric	4	1
58	OMCELLLEN	Numeric	4	1
59	OMCOLOUR	Character	5	
60	OMCELLSURF	Character	40	
61	OMANATNOTE	Character	130	
62	IMANTYPE	Numeric	1	
63	IMCELLSHAP	Character	50	
64	IMSEPTAVIS	Logical	1	
65	IMCLAMPS	Logical	1	
66	IMSEPTANOT	Character	30	
67	IMCELLWIDE	Numeric	4	1
68	IMCELLLEN	Numeric	4	1
69	IMCOLOUR	Character	5	
70	IMCELLSURF	Character	20	
71	IMANATNOTE	Character	80	
72	RZANAT	Character	65	
73	RZVESS	Character	50	
74	VESSDIAM	Numeric	4	1
75	EHHPSHAPE	Character	50	
76	EHSEPTAVIS	Logical	1	
77	EHCLAMPS	Logical	1	
78	EHSEPTANOT	Character	30	
79	EHDIAM	Numeric	4	1
80	EHWALLTHIK	Numeric	3	1
81	EHCOLOUR	Character	5	
82	EHSURF	Character	50	
83	EHRAM	Character	30	
84	EHPROXIMAL	Character	30	
85	EHDISTAL	Character	30	
86	EHJOINFREQ	Character	1	
87	EHJOINTYPE	Character	2	
88	EHANATNOTE	Character	80	
89	CY	Logical	1	

90	CYSHAPE	Character	45	
91	CYLEN	Numeric	5	1
92	CYWIDTH	Numeric	4	1
93	CYWALLTHIK	Numeric	3	1
94	CYCONT	Character	30	
95	CYCOLOUR	Character	5	
96	CYSURF	Character	30	
97	CYANATNOTE	Character	130	
98	SCLEROTIA	Logical	1	
99	MISCNOTES	Character	130	
**	Total	**	2407	

Field            Contents and codes

### **Identity**

EM            number of ectomycorrhizal type--all information in a record refers to this type

GENUS        known or suspected taxonomic identity

SPECIES

SN            serial number of soil core sample

DATE         date of description

NTIPS        number of root tips colonized in this core

TTOTAL      total number of tips colonized in all cores

CTOTAL      total number of cores in which this type was encountered (number of observations)

CLASS        degree to which the type has a consistent description and is distinguishable from other types: a=well-defined, consistent and common (>3 observations and >20 root tips) or else distinctive; b=tentative description based on few observations or tips, or a non-descript or variable type; c=poorly described or poorly developed type

**Morphology of tips**

RAM	type of ramification within system of mycorrhizal root tips: 1=simple, unramified; 2=monopodial pinnate, 3=monopodial pyramidal, 4=dichotomous, 5=irregularly pinnate, 6=coralloid, 7=tuberculate (Agerer 1993)
SYSLN	length of system of ectomycorrhizas (mm)
TIPDIAM	diameter of ectomycorrhizal root tips ( $\mu\text{m}$ )
AXDIAM	diameter of ectomycorrhizal axes of systems of root tips ( $\mu\text{m}$ )
TIPSHAPE	shape of tips: a=straight or nearly so, b=bent, c=tortuous
MORPHNOTE	notes on morphology
SEEMANTLE	whether or not the mantle is distinct from emanating hyphae and rhizomorphs: T=true, F=false
SEEROOT	whether or not the surface of the root cortex is visible through the mantle: T=true, F=false
MANTSURF	texture of the mantle surface: 10=smooth, 11=reticulate, 12=grainy or warty, 13=woolly, 14=cottony, 15=stringy, 16=short spiny, 17=long spiny (Agerer 1993), see morphnote for further description
COLOUR	singly or combined: e.g. lt-ye-br=light yellowish brown, dk-gr-bl=dark greenish blue, br+wh=brown and white, codes: wh or wt=white, br=brown, ye=yellow, bk=black, bl=blue, gr=green, gy=grey, pk=pink, pu=purple, or=orange, rd=red, bt=bright
NEWCOLOUR	colour of apices
OLDCOLOUR	colour of older parts
COLOURNOTE	notes on colour

**Morphology of rhizomorphs**

RZFREQ	frequency of rhizomorphs: a=abundant, c=common, o=occasional, n=none
RZOCCUR	location of occurrence of rhizomorphs on tips
RZCONNECT	how rhizomorphs are connected to the mantle: 18=at a restricted point, 19=at a larger area, growing off at narrow angles, 20=over large areas, forming hyphal fans (Agerer 1993)
RZCOLOUR	colour of rhizomorphs, see COLOUR for codes
RZDIAM	diameter of rhizomorphs ( $\mu\text{m}$ )
RZRAM	manner of ramification of rhizomorphs
RZXS	shape of rhizomorph in cross-section: r=round, f=flat, v=variable
RZSURF	nature of surface of rhizomorph: 21=interconnected filaments, 22=smooth, 23=hairy (Agerer 1993)
RZNOTE	notes on rhizomorph morphology

**Morphology of emanating hyphae**

EH	whether or not emanating hyphae can be seen at 40X: T=true, F=false.
EHDENSITY	density of emanating hyphae: a=abundant, c=common, o=occasional, n=none
EHOCCUR	location of occurrence of emanating hyphae
EHLPSHAPE	shape or appearance of emanating hyphae at low power (40X)
EHMORPNOTE	notes on the morphology of emanating hyphae at 40X

**Colour reactions to chemical reagents**

ETOH	colour reaction if any to immersion of tips in 70% ethanol for 5 minutes
FES04	colour reaction if any to immersion of tips in

	10% ferrous sulphate for 5 minutes
KOH	colour reaction if any to immersion of tips in 15% potassium hydroxide for 5 minutes
LACTICACID	colour reaction if any to immersion of tips in 85% lactic acid for 5 minutes
MELTZERS	colour reaction if any to immersion of tips in Meltzer's reagent for 5 minutes
SULFOVAN	colour reaction if any to immersion of tips in sulfo-vanillin for 5 minutes

### **Autofluorescence**

AF254	colour if any of autofluorescence of whole ectomycorrhizas under 254 nm ultra-violet light: n=none, ye=yellow, bt-ye=bright yellow
AF366	colour if any of autofluorescence of whole ectomycorrhizas under 366 nm ultra-violet light: see AF254

### **Anatomy of laticiferous hyphae**

LACT	whether or not laticiferous hyphae are present in the mantle: T=true, F=false
LACTDIST	distribution of laticiferous hyphae in mantle
LACTDIAM	diameter of laticiferous hyphae ( $\mu\text{m}$ )
LACTRAM	nature of ramification of laticiferous hyphae
LACTCONT	contents of laticiferous hyphae
LACTCOLOUR	colour of laticiferous hyphae: see COLOUR for codes
LACTSEPTA	nature of septa of laticiferous hyphae

### **Anatomy of outer mantle**

OMANTYPE	type of arrangement of hyphae or cells of outermost layer of mantle: 1=felt prosenchyma, 2=net prosenchyma, 3=net synenchyma,
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- 4=interlocking irregular synenchyma (jigsaw or epidermioid, 5=non-interlocking irregular synenchyma (round cells), 6=regular synenchyma (polygonal cells) (Ingleby et al. 1990)
- OMCELLSHAP shape of cells of outer mantle
- OMSEPTAVIS whether or not septa can be seen in the outermost layer of the mantle: T=true, F=false
- OMCLAMPS whether or not clamp connections are present in the outermost layer of the mantle: T=true, F=false
- OMSEPTANOT notes about the septa or clamps in the outermost layer of the mantle
- OMCELLWIDE width of hyphae or cells in the outermost layer of the mantle ( $\mu\text{m}$ )
- OMCELLLEN length of the cells in the outermost layer of the mantle ( $\mu\text{m}$ )
- OMCOLOUR colour of the hyphae or cells in the outermost layer of the mantle: see COLOUR for codes
- OMCELLSURF nature of the surface of cells or hyphae in the outermost layer of the mantle: sm=smooth, orn=ornamented, xtls=crystals
- OMANATNOTE notes on the anatomy of the outermost layer of the mantle

#### **Anatomy of inner mantle**

- IMANTYPE type of arrangement of hyphae or cells of innermost layer of the mantle: see omantype for codes
- IMCELLSHAP shape of cells or hyphae of innermost layer of mantle
- IMSEPTAVIS whether or not septa are visible in the innermost layer of the mantle: T=true, F=false
- IMCLAMPS whether or not clamp connections are present in

- the innermost layer of the mantle: T=true,  
F=false
- IMSEPTANOT notes on the nature of septa in the innermost  
layer of the mantle
- IMCELLWIDE width of hyphae or cells in the innermost layer  
of the mantle ( $\mu\text{m}$ )
- IMCELLLEN length of the cells in the innermost layer of  
the mantle ( $\mu\text{m}$ )
- IMCOLOUR colour of cells or hyphae in the innermost  
layer of the mantle: see COLOUR for codes
- IMCELLSURF nature of the surface of cells or hyphae in the  
innermost layer of the mantle: see OMCELLSURF  
for abbreviations
- IMANATNOTE notes on the anatomy of the innermost layer of  
the mantle

#### **Anatomy of rhizomorphs**

- RZANAT features of the anatomy of rhizomorphs
- RZVESS features of vessel-like hyphae of rhizomorphs
- VESSDIAM diameter of vessel-like hyphae of rhizomorphs  
( $\mu\text{m}$ )

#### **Anatomy of emanating hyphae**

- EHHPSHAPE shape of emanating hyphae at high power (1000X)
- EHSEPTAVIS whether or not septa are visible in emanating  
hyphae: T=true, F=false
- EHCLAMPS whether or not clamp connections are present on  
emanating hyphae: T=true, F=false
- EHSEPTANOT notes on the features of the septa of emanating  
hyphae
- EHDIAM diameter of emanating hyphae ( $\mu\text{m}$ )
- EHWALLTHIK thickness of walls of emanating hyphae ( $\mu\text{m}$ )
- EHCOLOUR colour of emanating hyphae: see COLOUR for

	codes
EHSURF	nature of surface of emanating hyphae: see OMCELLSURF for abbreviations
EHRAM	nature of ramification of emanating hyphae
EHPROXIMAL	nature of proximal region of emanating hyphae
EHDISTAL	nature of distal region and tips of emanating hyphae
EHJOINREQ	frequency of anastomoses of emanating hyphae
EHJOINTYPE	type of anastomoses of emanating hyphae: see Agerer (1993)
EHAJATNOTE	notes on the anatomy of emanating hyphae

#### **Anatomy of cystidia or setae**

CY	whether or not cystidia or setae are present: T=true, F=false
CYSHAPE	shape of cystidia or setae
CYLEN	length of cystidia or setae ( $\mu\text{m}$ )
CYWIDTH	width of cystidia or setae ( $\mu\text{m}$ )
CYWALLTHIK	thickness of walls of cystidia or setae ( $\mu\text{m}$ )
CYCONT	contents of cystidia or setae
CYCOLOUR	colour of cystidia or setae: see COLOUR for codes
CYSURF	nature of surface of cystidia or setae: see OMCELLSURF for abbreviations
CYANATNOTE	notes on the anatomy of cystidia or setae
SCLEROTIA	whether or not sclerotia are present: T=true, F=false
MISCNOTES	miscellaneous notes on the description and distinguishing features

## 2. Contents of database

EM:1 GENUS:*Cenococcum* SPECIES:*geophilum* SN:1  
 DATE:05/08/92 NTIPS:130 TTOTAL:4262 CTOTAL:75 CLASS:a  
 RAM:1 SYLEN:13 mm TIPDIAM:750 µm AXDIAM:750 µm  
 TIPSHAPE:a MORPHNOTE:diameters vary widely, surface shiny,  
 cystidia often densest subapically SEEMANTLE?.T.  
 SEEROOT?.F. MANTSURF:17 COLOUR:bk NEWCOLOUR:bk  
 OLDLOUR:bk RZFREQ:n EH?.T. ETOH:n FESO4:n KOH:n  
 LACTICACID:n MELTZERS:n SULFOVAN:n AF254:n AF366:n  
 LACT? .F. OMANTYPE:3 OMCELLSHAP:long rectangular  
 OMSEPTAVIS?.F. OMCOLOUR:brown IMANTYPE:0 IMSEPTAVIS?.F.  
 EHHPSHAPE:straight or slightly curved EHSEPTAVIS?.F.  
 EHCLOUR:br EHANATNOTE:thin CY?.T. CYSHAPE:straight  
 CYCOLOUR:br CYSURF:smooth to slightly rough SCLEROTIA?.T.

EM:2 GENUS:*Hysterangium* SN:5 DATE:05/23/92 NTIPS:40  
 TTOTAL:1650 CTOTAL:37 CLASS:a RAM:2 SYLEN:8 mm  
 TIPDIAM:600 µm AXDIAM:600 µm TIPSHAPE:b  
 MORPHNOTE:cheesecloth texture SEEMANTLE?.T. SEEROOT?.F.  
 MANTSURF:11 COLOUR:wt NEWCOLOUR:wt OLDLOUR:wt  
 COLOURNOTE:yellowish in spots mixed with em3 hyphae,  
 off-white often, not silvery RZFREQ:a RZOCCUR:everywhere,  
 especially at ends, branches and bends RZCONNECT:19  
 RZCOLOUR:wt RZDIAM:500 µm RZRAM:often, into small strands  
 RZXS:f RZSURF:23 RZNOTE:rzdiam is upper limit, many  
 smaller ones, rzram not as connected as 21 EH?.T.  
 EHDENSITY:f EHOCCUR:everywhere, but especially near  
 rhizomorphs EHLPSHAPE:wispy EHMORPNOTE:intergrade with  
 hyphae, wispy curving back towards root ETOH:some clearing  
 FESO4:n KOH:cleared LACTICACID:partly cleared  
 MELTZERS:brownish yellow-yellowish SULFOVAN:pink-orange  
 root (transparent mantle) AF254:n AF366:n LACT? .F.

OMANTYPE:1 OMSEPTAVIS?.T. OMCLAMPS?.F. OMCELLWIDE:2 µm  
 OMCOLOUR:none OMCELLSURF:crystals 30 deg to axis, none to  
 many OMANATNOTE:spaghetti like, sn74: width 2.5-3, loose  
 mantle IMANTYPE:1 IMCELLSHAP:as outer? IMSEPTAVIS?.T.  
 IMCLAMPS?.F. IMCELLWIDE:2 µm IMCOLOUR:n IMCELLSURF:as  
 outer? IMANATNOTE:sn74: same as outer RZANAT:gradually  
 disperses at narrow angle, few eh RZVESS:no  
 EHHPSHAPE:straight EHSEPTAVIS?.T. EHCLAMPS?.F. EHDIAM:3  
 µm EHCLOUR:none EHSURF:elongated xtls none to many  
 EHRAM:occasional 90 deg EHDISTAL:smooth narrow, wiggly  
 EHJOINFREQ:c EHJOINTYPE:d2 EHANATNOTE:h-shaped  
 anastomoses, fewer xtls than in rz and mantle, xtls come  
 off CY?.F. SCLEROTIA?.F.

EM:3 GENUS:*Piloderma* SPECIES:*fallax* SN:28 DATE:12/02/93  
 NTIPS:29 TTOTAL:632 CTOTAL:21 CLASS:a RAM:5 SYLEN:18  
 mm TIPDIAM:500 µm AXDIAM:550 µm TIPSHAPE:b  
 MORPHNOTE:cheesecloth texture SEEMANTLE?.T. SEEROOT?.F.  
 MANTSURF:11 COLOUR:ye NEWCOLOUR:ye OLDLOUR:ye  
 COLOURNOTE:slightly brownish, bright RZFREQ:a  
 RZOCCUR:everywhere, especially at on tips RZCONNECT:19  
 RZCOLOUR:ye RZDIAM:600 µm RZRAM:frequent at narrow angle  
 RZXS:r RZSURF:21 RZNOTE:many hyphae coming off, many  
 about 100µm thick EH?.T. EHDENSITY:a EHOCCUR:everywhere  
 EHLPSHAPE:bit wobbly, not straight or curved  
 EHMORPNOTE:form small strands ETOH:n FESO4:n KOH:white  
 (very slight yellow), slightly bluish (root) LACTICACID:n  
 MELTZERS:n SULFOVAN:n AF254:n AF366:n LACT? .F.  
 OMANTYPE:1 OMSEPTAVIS?.T. OMCLAMPS?.F. OMSEPTANOT:few  
 OMCELLWIDE:3 µm OMCOLOUR:ye-br OMCELLSURF:finely  
 ornamented OMANATNOTE:sn28: width 2.5-3 IMANTYPE:2  
 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:2 µm IMCOLOUR:ye  
 IMCELLSURF:smooth IMANATNOTE:some modification of hyphal

shape, more rounded near septa RZANAT:linear parallel  
 hyphae RZVESS:no EHHPSHAPE:straight EHSEPTAVIS?.T.  
 EHCLAMPS?.F. EHSEPTANOT:few EHCOLOUR:ye EHSURF:finely  
 ornamented, round dots EHRAM:90 deg EHJOINRFREQ:a  
 EHJOINTYPE:d2 EHANATNOTE:sn74: ram 60 deg CY?.F.  
 SCLEROTIA?.T.

EM:4 SN:1 DATE:05/10/92 NTIPS:60 TTOTAL:60 CTOTAL:2  
 CLASS:c RAM:5 SYSLLEN:10 mm TIPDIAM:750 µm AXDIAM:750 µm  
 TIPSHAPE:a MORPHNOTE:surface smooth-stringy SEEMANTLE?.T.  
 SEEROOT?.T. MANTSURF:10 COLOUR:lt-br NEWCOLOUR:lt-br  
 OLDCCOLOUR:lt-br COLOURNOTE:transparent mantle slightly  
 whitish RZFREQ:n EH?.F. KOH:dark brown LACT?.F.  
 OMANTYPE:2 OMCELLSHAP:some bulbous or very round at tips  
 OMSEPTAVIS?.T. OMCLAMPS?.F. OMCELLWIDE:5 µm  
 OMCOLOUR:none OMCELLSURF:smooth OMANATNOTE:bulbous tips  
 10-15 µm IMANTYPE:0 IMSEPTAVIS?.F. EHHPSHAPE:in mantle,  
 bulbous tips EHSEPTAVIS?.T. EHCLAMPS?.F. EHDIAM:3 µm  
 EHCOLOUR:none EHSURF:smooth EHRAM:elbows EHDISTAL:not  
 narrowed CY?.F. SCLEROTIA?.F. MISCNOTES:rare, poorly  
 developed mantle, perhaps the beginning or end of another  
 type

EM:5 SN:34 DATE:01/29/93 NTIPS:43 TTOTAL:48 CTOTAL:4  
 CLASS:a RAM:2 SYSLLEN:10 mm TIPDIAM:620 µm AXDIAM:700 µm  
 TIPSHAPE:b MORPHNOTE:tipshape b-c, mantsurf 11-12,  
 icing-sugar-coated or snowy, cortex shows thru a bit  
 SEEMANTLE?.T. SEEROOT?.F. MANTSURF:12 COLOUR:wt  
 NEWCOLOUR:wt OLDCCOLOUR:wt COLOURNOTE:bright, slightly  
 greenish blue staining and small patches, sn99:no blue  
 RZFREQ:c RZOCUR:anywhere, common to abundant  
 RZCONNECT:18 RZCOLOUR:wt RZDIAM:110 µm RZRAM:60-90  
 degrees RZXS:r RZSURF:23 RZNOTE:blue stains, not as

distinct rzconnect as 18, rzsrf not as hairy as 23 EH?.T.  
 EHDENSITY:c EHOCCUR:all over EHLPSHAPE:wispy ETOH:n  
 FESO4:n KOH:n LACTICACID:n MELTZERS:n SULFOVAN:purple  
 after rinsing (not sn99) AF254:none AF366:none LACT? .F.  
 OMANTYPE:2 OMCELLSHAP:convoluted, bit like staghorns of  
*By. sporidia* (Zak) OMSEPTAVIS?.T. OMCLAMPS?.F.  
 OMCELLWIDE:3  $\mu$ m OMCOLOUR:none OMCELLSURF:smooth  
 OMANATNOTE:much granules, hard to see, hyphae  
 2,2,2,2.5,2.5,4 wide IMANTYPE:3 IMCELLSHAP:linear  
 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:3  $\mu$ m  
 IMCOLOUR:none IMCELLSURF:smooth IMANATNOTE:v. thin  
 walled, hyaline, hard to see RZANAT:outer layer of highly  
 convoluted hyphae 1-1.5  $\mu$ m EHSEPTAVIS?.F. CY?.F.  
 SCLEROTIA?.F.

EM:6 SN:6 DATE:02/16/93 NTIPS:108 TTOTAL:325 CTOTAL:11  
 CLASS:a RAM:2 SYLEN:21 mm TIPDIAM:550  $\mu$ m AXDIAM:650  $\mu$ m  
 TIPSHAPE:b MORPHNOTE:brittle mantle, soil particles stick  
 on, matte smooth mostly, sn91:slightly fuzzy with age  
 SEEMANTLE?.T. SEEROOT?.T. MANTSURF:10 COLOUR:or-br  
 NEWCOLOUR:ye-br OLDLOUR:lt-br RZFREQ:n EH?.F. ETOH:n  
 FESO4:grey KOH:grey LACTICACID:n MELTZERS:n SULFOVAN:n  
 AF254:n AF366:n LACT? .F. OMANTYPE:2 OMCELLSHAP:some  
 rounded, hyphae hard to see OMSEPTAVIS?.T. OMCLAMPS?.F.  
 OMCELLWIDE:3  $\mu$ m OMCELLLEN:12  $\mu$ m OMCOLOUR:none  
 OMCELLSURF:smooth OMANATNOTE:hyphae 1.4-4  $\mu$ m, hard to see  
 hyphae, network, sn91: oil droplets in hyphae, some  
 granular contents IMANTYPE:3 IMSEPTAVIS?.T. IMCLAMPS?.F.  
 IMCELLWIDE:3  $\mu$ m IMCOLOUR:none IMCELLSURF:smooth  
 IMANATNOTE:hard to see EHLPSHAPE:kinky EHSEPTAVIS?.T.  
 EHCLAMPS?.T. EHSEPTANOT:clamped? eh of same fungus?  
 EHDIAM:3  $\mu$ m EHANATNOTE:sn41:1.5-2.5 wide CY?.F.  
 SCLEROTIA?.F.

EM:7 GENUS:Rhizopogon SPECIES:vinicolor SN:14  
 DATE:05/23/92 NTIPS:287 TTOTAL:1790 CTOTAL:25 CLASS:a  
 RAM:7 SYLEN:5 mm TIPDIAM:500  $\mu$ m TIPSHAPE:b  
 MORPHNOTE:surface silvery-felty, peridium surface like  
 rhizomorph surface SEEMANTLE?.T. SEEROOT?.F. MANTSURF:11  
 COLOUR:wt NEWCOLOUR:wt OLDLOUR:br-wt COLOURNOTE:apices  
 a bit greyish-translucent sometimes, some brown hyphae may  
 stick on RZFREQ:a RZOCCUR:on tubercle surface, 3-8 per  
 tubercle RZCONNECT:18 RZCOLOUR:br-sv RZDIAM:260  $\mu$ m  
 RZRAM:frequent y-shaped RZXS:r RZSURF:22 RZNOTE:cryotome  
 xs @ UEC: some hyaline larger hyphae inside EH?.F.  
 ETOH:when rinsed, clearing of mantle FESO4:n  
 KOH:darkening LACTICACID:n MELTZERS:light brown after  
 rinsing SULFOVAN:dark brown after rinsing AF254:none  
 AF366:none LACT? .F. OMANTYPE:3 OMCELLSHAP:parallel  
 cells OMSEPTAVIS?.T. OMCLAMPS?.F. OMCELLWIDE:5  $\mu$ m  
 OMCELLLEN:25  $\mu$ m OMCOLOUR:none OMCELLSURF:smooth  
 OMANATNOTE:largest cells outermost IMANTYPE:?  
 IMSEPTAVIS?.F. IMCELLWIDE:2  $\mu$ m RZANAT:thin dark outer  
 hyphae with green xtls on surface RZVESS:hyaline inner  
 hyphae, including vessels EHHPSHAPE:bit curved with elbows  
 EHSEPTAVIS?.F. EHSEPTANOT:no septa observed EHDIAM:2  $\mu$ m  
 EHCLOUR:br EHSURF:some small green xtls (rz) EHRAM:rare  
 60 degrees EHDISTAL:narrowed, round to 1-2  $\mu$ m  
 EHJOINFREQ:n EHANATNOTE:description of brown felty  
 mycelium assoc. with peridium, with elbows, sn16: 2.8  
 CY?.F. SCLEROTIA?.F.

EM:8 SN:68 DATE:06/17/93 NTIPS:23 TTOTAL:46 CTOTAL:5  
 CLASS:a RAM:2 SYLEN:7 mm TIPDIAM:670  $\mu$ m TIPSHAPE:b  
 MORPHNOTE:snowy surface felty SEEMANTLE?.T. SEEROOT?.F.  
 MANTSURF:11 COLOUR:wt NEWCOLOUR:wt OLDLOUR:wt  
 RZFREQ:a RZOCCUR:all over, esp at tips RZCONNECT:20

RZCOLOUR:wt RZDIAM:500 µm RZRAM:diffuse, many smaller eg  
 50µm rz RZXS:f RZSURF:23 RZNOTE:intergrades with eh  
 EH?.T. EHDENSITY:a EHOCUR:all over EHLPSHAPE:cottony  
 EHMORPNOTE:loosely organized into eh in places ETOH:none  
 FESO4:none KOH:none LACTICACID:none MELTZERS:none  
 SULFOVAN:none AF254:none AF366:none LACT? .F.  
 OMANTYPE:2 OMCELLSHAP:network OMSEPTAVIS?.T.  
 OMCLAMPS?.T. OMSEPTANOT:few clampless OMCELLWIDE:5 µm  
 OMCOLOUR:none OMCELLSURF:smooth OMANATNOTE:looks type1  
 with eh 2.5,6,4.5,4,5,5.5,3.5 wide, type 2 check sn73  
 IMANTYPE:3 IMCELLSHAP:some round, some triangular, some  
 long like type3 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:7  
 µm IMCELLLEN:15 µm IMCOLOUR:none IMCELLSURF:smooth  
 IMANATNOTE:size eg 5x22, 9x9, 12x7, sn73: type 3-6, sn104:  
 type 3-5 RZANAT:parallel hyphae EHHPSHAPE:slightly curved  
 EHSEPTAVIS?.T. EHCLAMPS?.T. EHDIAM:4 µm EHCOLOUR:none  
 EHSURF:smooth EHRAM:60 degrees EHDISTAL:rounded  
 EHJOINFREQ:o EHJOINTYPE:a1 EHANATNOTE:slight constriction  
 at septa (check sn104) CY?.F. SCLEROTIA?.F.

EM:9 SN:5 DATE:05/20/92 NTIPS:30 TTOTAL:637 CTOTAL:3  
 CLASS:a RAM:2 SYLEN:5 mm TIPDIAM:600 µm AXDIAM:600 µm  
 TIPSHAPE:b SEEMANTLE?.T. SEEROOT?.F. MANTSURF:12  
 COLOUR:dk-br NEWCOLOUR:dk-br OLDLOUR:dk-br  
 COLOURNOTE:lighter at tip, darker on older parts RZFREQ:n  
 EH?.F. ETOH:darker after rinsing FESO4:n KOH:black  
 LACTICACID:darker after rinsing MELTZERS:darker after  
 rinsing SULFOVAN:n AF254:none AF366:none LACT? .F.  
 OMANTYPE:2 OMCELLSHAP:curved, some enlarged (sn6,91)  
 OMSEPTAVIS?.T. OMCLAMPS?.T. OMCELLWIDE:5 µm  
 OMCELLSURF:smooth to finely and densely dotted  
 OMANATNOTE:type 3 slightly below mantle, sn6 dense and  
 brittle, sn91 swollen to 8, IMANTYPE:3 IMSEPTAVIS?.T.

IMCLAMPS?.F. IMCELLWIDE:4 µm IMCOLOUR:br IMCELLSURF:sm  
 IMANATNOTE:obs from sn91 EHSEPTAVIS?.T. EHCLAMPS?.T.  
 EHDIAM:4 µm EHCOLOR:br EHSURF:smooth sn91: some finely  
 dotted EHRAM:sn91:90 deg, CY?.F. SCLEROTIA?.F.

EM:10 SN:35 DATE:02/08/93 NTIPS:325 TTOTAL:409  
 CTOTAL:3 CLASS:a RAM:5 SYLEN:0 mm TIPDIAM:0 µm  
 TIPSHAPE:b MORPHNOTE:tipshape sometimes c SEEMANTLE?.T.  
 SEEROOT?.F. MANTSURF:11 COLOUR:wt NEWCOLOUR:wt  
 OLDCOLOUR:sv-gy COLOURNOTE:root cortex shows thru a bit,  
 sn46: silvery; brighter fluor. at 366 (check sn46)  
 RZFREQ:a RZOCUR:all over RZCONNECT:0 RZCOLOUR:wt  
 RZXS:f RZSURF:22 EH?.F. ETOH:partial clearing FESO4:n  
 KOH:n LACTICACID:n MELTZERS:DARK!, even Rhizomorphs  
 SULFOVAN:n AF254:ye AF366:bt-ye LACT? .F. OMANTYPE:1  
 OMSEPTAVIS?.T. OMCLAMPS?.T. OMSEPTANOT:but mostly  
 clampless OMCELLWIDE:4 µm OMCOLOUR:none  
 OMCELLSURF:smooth OMANATNOTE:can see types 2,3 and 4 with  
 depth,3,4,5,5 hypwide IMANTYPE:3 IMCELLSHAP:varied  
 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:5 µm IMCELLLEN:14  
 µm IMCOLOUR:none IMCELLSURF:smooth IMANATNOTE:parallel  
 hyphae in layer, layers with perpendicular hyphae, type 4-5  
 in places, EHSEPTAVIS?.T. EHCLAMPS?.T. EHSEPTANOT:small  
 bumpy clamps EHDIAM:5 µm EHCOLOR:none EHSURF:smooth  
 EHRAM:30 deg at clamp or 90 deg EHDISTAL:not narrowed  
 CY?.F. SCLEROTIA?.F. MISCNOTES:resembles em16,11 but is  
 fluorescent and has more abundant rz and is slightly  
 yellowish

EM:11 SN:69 DATE:07/14/93 NTIPS:75 TTOTAL:158 CTOTAL:3  
 CLASS:b RAM:5 SYLEN:7 mm TIPDIAM:480 µm AXDIAM:480 µm  
 TIPSHAPE:c MORPHNOTE:tipshape c sn112, not much of cortex  
 visible sn112, SEEMANTLE?.T. SEEROOT?.T. MANTSURF:11

COLOUR:wt NEWCOLOUR:wt OLDCOLOUR:wt COLOURNOTE:brown  
 shows thru in places, snowy, older ones with black cortex  
 (carbonizing) RZFREQ:a RZOCCUR:subapical RZCONNECT:19  
 RZCOLOUR:wt RZDIAM:110  $\mu$ m RZRAM:sn112:30 deg and less  
 RZXS:r RZSURF:22 RZNOTE:frayed look, 50-80 wide sn112  
 EH?.T. EHDENSITY:a EHOCCUR:all over EHLPSHAPE:wispy  
 EHMORPNOTE:comes away with soil, soil holds onto eh  
 ETOH:none FESO4:none KOH:none LACTICACID:none  
 MELTZERS:none SULFOVAN:none AF254:none AF366:none LACT?  
 .F. OMANTYPE:1 OMSEPTAVIS?.T. OMCLAMPS?.T.  
 OMSEPTANOT:clamps hemispherical same size as hyphae  
 OMCELLWIDE:3  $\mu$ m OMCOLOUR:none OMCELLSURF:smooth  
 OMANATNOTE:hyphae size range: 2,2.5,3,3,3,2.5, sn112:  
 type2, thin-walled hard to see hyphae IMANTYPE:3  
 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:3  $\mu$ m IMCELLEN:10  
 $\mu$ m IMCOLOUR:none IMCELLSURF:smooth IMANATNOTE:someplaces  
 approaching type 4, 3,2.5,4,2.5,4,3,3,2,3,3 widths  
 RZANAT:sn112: some weaving, clamped hyphae, central hyphae  
 to 7  $\mu$ m EHSEPTAVIS?.T. EHCLAMPS?.T. EHDIAM:3  $\mu$ m  
 EHCLOUR:none EHSURF:smooth EHRAM:60 deg EHDISTAL:blunt  
 EHANATNOTE:widths: 2.5,3,2.5,2,2.5,3 CY?.F. SCLEROTIA?.F.  
 MISCNOTES:fairly non-distinctive, maybe same as em16

EM:12 SN:53 DATE:05/27/93 NTIPS:67 TTOTAL:436 CTOTAL:5  
 CLASS:a RAM:2 SYSLEN:9 mm TIPDIAM:660  $\mu$ m TIPSHAPE:a  
 MORPHNOTE:white (warts) surface texture on most parts, some  
 smooth, coralloid(6)(sn36) SEEMANTLE?.T. SEEROOT?.T.  
 MANTSURF:12 COLOUR:lt-br NEWCOLOUR:lt-gy OLDCOLOUR:br  
 COLOURNOTE:older parts wt-gy sometimes, gy due to dark  
 tannin layer RZFREQ:o RZOCCUR:all over RZCONNECT:19  
 RZCOLOUR:wt RZDIAM:65  $\mu$ m RZRAM:quickly ramifies into  
 small strands throughout humus RZXS:f RZSURF:21  
 RZNOTE:lots of eh, round to flat rz, small weftlike in

humus (sn14) EH?.F. ETOH:n FESO4:n KOH:cleared a bit  
 LACTICACID:shrivelled MELTZERS:n SULFOVAN:mantle stains  
 pink in patchy way AF254:n AF366:n LACT? .F. OMATYPE:2  
 OMSEPTAVIS?.T. OMCLAMPS?.F. OMCELLWIDE:5 µm OMCELLLEN:12  
 µm OMCOLOUR:none OMCELLSURF:smooth OMANATNOTE:type 5  
 parts (eg10x10), hard to see, fungal warts, sn14: type 3-4,  
 sn68: type 5 IMATYPE:2 IMSEPTAVIS?.T. IMCLAMPS?.F.  
 IMCELLWIDE:3 µm IMCOLOUR:none IMCELLSURF:smooth  
 IMANATNOTE:type 3 (sn68r) EHHPSHAPE:wiggly to straight  
 EHSEPTAVIS?.F. EHDIAM:3 µm EHCOLOUR:none EHSURF:smooth  
 EHRAM:smooth junction EHDISTAL:not narrowed CY?.F.  
 SCLEROTIA?.F.

EM:13 GENUS:*Lactarius* SPECIES:*deliciosus* SN:11  
 DATE:05/29/92 NTIPS:140 TTOTAL:1007 CTOTAL:18 CLASS:a  
 RAM:2 SYLEN:40 mm TIPDIAM:540 µm AXDIAM:600 µm  
 TIPSHAPE:a MORPHNOTE:surface someplaces slightly felty  
 SEEMANTLE?.T. SEEROOT?.T. MANTSURF:10 COLOUR:lt-br  
 NEWCOLOUR:lt-br OLDCOLOUR:dk-tu COLOURNOTE:some tips and  
 joints and breaks with turquoise patches or green patches,  
 RZFREQ:o RZOCCUR:2 observed in midway between branch and  
 very tip RZCONNECT:18 RZCOLOUR:gr RZDIAM:60 µm RZRAM:30  
 degrees, at first into two, later into many RZXS:r  
 RZSURF:22 RZNOTE:bright green beautiful, stand out in  
 soil, diam 110 sn38 EH?.F. ETOH:n FESO4:n KOH:darkened  
 LACTICACID:shrivelled(collapsed cortex)  
 MELTZERS:shrivelled SULFOVAN:dark and shrivelled AF254:n  
 AF366:n LACT? .T. LACTDIST:midlayer or inner layer, sn38  
 top to bottom LACTDIAM:6 µm LACTRAM:smooth 60deg widening  
 LACTCONT:granular or not, ye or LACTCOLOUR:ye LACTSEPTA:?  
 OMATYPE:3 OMCELLSHAP:wiggly OMSEPTAVIS?.T. OMCLAMPS?.F.  
 OMSEPTANOT:curved septa OMCELLWIDE:3 µm OMCOLOUR:none  
 OMCELLSURF:smooth OMANATNOTE:hyphae sometimes green, some

2  $\mu\text{m}$ , thin mantle IMATYPE:3 IMSEPTAVIS?.F. IMCELLWIDE:4  $\mu\text{m}$  IMANATNOTE:some large 6-10  $\mu\text{m}$  cells, sometimes type 2, cells sometimes 3  $\mu\text{m}$  RZANAT:linear, thick green outer layer, lact. inside RZVESS:some EHSEPTAVIS?.F. CY?.F. SCLEROTIA?.F.

EM:14 SN:61 DATE:06/07/93 NTIPS:24 TTOTAL:201 CTOTAL:9  
 CLASS:a RAM:2 SYSLIN:15 mm TIPDIAM:608  $\mu\text{m}$  AXDIAM:710  $\mu\text{m}$   
 TIPSHAPE:b MORPHNOTE:some straight tips, tightly grouped tips, dark bumps or warts on surface (check sn115)  
 SEEMANTLE?.T. SEEROOT?.F. MANTSURF:12 COLOUR:br  
 NEWCOLOUR:br OLDLOUR:dk-br COLOURNOTE:patches of dark pigment in mantle, esp on older parts, sn84 dk-br, other sn lt-br RZFREQ:n EH?.F. KOH:darkened  
 LACTICACID:shrivelled MELTZERS:shrivelled SULFOVAN:n  
 AF254:n AF366:n LACT? .F. OMATYPE:5  
 OMCELLSHAP:roundish OMSEPTAVIS?.F. OMCELLWIDE:15  $\mu\text{m}$   
 OMCELLLEN:28  $\mu\text{m}$  OMCOLOUR:br OMCELLSURF:smooth  
 OMANATNOTE:walls 1-2(5)  $\mu\text{m}$  thick, width  
 30x15, 23x13, 45x15, 25x27, 40x12, 30x6 IMATYPE:3  
 IMCELLSHAP:some quadrangular some like type 4  
 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:5  $\mu\text{m}$  IMCELLLEN:18  $\mu\text{m}$   
 IMCOLOUR:br IMCELLSURF:smooth IMANATNOTE:few places type 3 thin walled, sn115 type 3-4. sizes  
 20x4, 20x7, 16x5, 20x5, 19x5 EHHPSHAPE:bumpy EHSEPTAVIS?.T.  
 EHCLAMPS?.F. EHDIAM:5  $\mu\text{m}$  EHWALLTHIK:1  $\mu\text{m}$  EHCLOUR:br  
 EHSURF:smooth EHDISTAL:bit thinner, less colour  
 EHANATNOTE:some thin walled CY?.T. CYSHAPE:stubby horns, triangular  
 CYLEN:13  $\mu\text{m}$  CYWIDTH:10  $\mu\text{m}$  CYWALLTHIK:4  $\mu\text{m}$   
 CYCOLOUR:br CYSURF:smooth CYANATNOTE:some black, walls 1-5 $\mu\text{m}$  can fill cy, 2.5  $\mu\text{m}$  at tip SCLEROTIA?.F.

EM:15 SN:14 DATE:06/10/92 NTIPS:60 TTOTAL:60 CTOTAL:1

CLASS:a RAM:2 SYSLEN:22 mm TIPDIAM:700  $\mu$ m AXDIAM:800  $\mu$ m  
 TIPSHAPE:b MORPHNOTE:surface finely woolly SEEMANTLE?.T.  
 SEEROOT?.F. MANTSURF:15 COLOUR:lt-ye NEWCOLOUR:  
 OLDLOUR:gy RZFREQ:n EH?.T. EHDENSITY:c EHOCCUR:all  
 over EHLPSHAPE:wispy EHMORPNOTE:v. short LACT? .F.  
 OMANTYPE:1 OMSEPTAVIS?.T. OMCLAMPS?.T. OMSEPTANOT:some  
 with clamps, some without OMCELLWIDE:3  $\mu$ m OMCELLLEN:22  $\mu$ m  
 OMCOLOUR:none OMCELLSURF:smooth IMANTYPE:3  
 IMCELLSHAP:perpendicular layers gives appearance of type 6  
 IMSEPTAVIS?.T. IMCLAMPS?.F. IMSEPTANOT:not noted  
 EHHPSHAPE:wispy, short, like surface hyphae EHSEPTAVIS?.F.  
 EHDIAM:3  $\mu$ m CY?.T. CYSHAPE:various, knife, spike  
 CYLEN:12  $\mu$ m CYCONT:clear CYCOLOUR:none CYSURF:smooth  
 SCLEROTIA?.F.

EM:16 SN:15 DATE:05/12/92 NTIPS:133 TTOTAL:488  
 CTOTAL:9 CLASS:b RAM:5 SYSLEN:10 mm TIPDIAM:450  $\mu$ m  
 AXDIAM:550  $\mu$ m TIPSHAPE:c MORPHNOTE:tips not as beaded as  
 in fig 8c, irreg. ram.--freq. singly bifurcate  
 SEEMANTLE?.T. SEEROOT?.T. MANTSURF:11 COLOUR:wt  
 NEWCOLOUR:wt OLDLOUR:wt COLOURNOTE:silvery w brown  
 patches, apices often brown RZFREQ:a ZOCCUR:all over,not  
 esp at tip but there sometimes RZCONNECT:19 RZCOLOUR:wt  
 RZDIAM:70  $\mu$ m RZRAM:gradual into many strands, permeates  
 humus RZXS:v RZSURF:23 RZNOTE:rzdiam upto 125, rzsrf  
 not as extreme as fig 23 EH?.T. EHDENSITY:f EHOCCUR:all  
 over EHLPSHAPE:gently curved ETOH:some clearing FESO4:n  
 KOH:clearing and root darkening LACTICACID:n MELTZERS:n  
 SULFOVAN:cleared AF254:n AF366:n LACT? .F. OMANTYPE:1  
 OMSEPTAVIS?.T. OMCLAMPS?.T. OMSEPTANOT:not noted  
 OMCELLWIDE:4  $\mu$ m OMCELLLEN:15  $\mu$ m OMCOLOUR:none  
 OMCELLSURF:smooth OMANATNCTE:some clampless septa, very  
 hyaline, hard to see, sn59,82: hyphae 3-5 wide IMANTYPE:3

IMSEPTAVIS?.T. IMCLAMPS?.F. IMSEPTANOT:not noted  
 IMCELLWIDE:4 µm IMCELLLEN:14 µm IMCOLOUR:none  
 IMCELLSURF:smooth IMANATNOTE:type more irregular than fig  
 2.3., sn59,82: cells 3-6 wide RZANAT:few em, parallel  
 hyphae EHHPSHAPE:straight EHSEPTAVIS?.T. EHCLAMPS?.T.  
 EHDIAM:4 µm EHCOLOUR:none EHSURF:smooth EHRAM:narrow  
 angle EHANATNOTE:few swellings at septa, forms fuzzy  
 masses (sn 621) CY?.F. SCLEROTIA?.F. MISCNOTES:variable  
 grouping, may be several taxa

EM:17 SN:15 DATE:06/14/92 NTIPS:34 TTOTAL:65 CTOTAL:2  
 CLASS:b RAM:2 SYLEN:23 mm TIPDIAM:580 µm AXDIAM:580 µm  
 TIPSHAPE:a MORPHNOTE:ram a bit like 5, very few curved  
 tips, transparent mantle SEEMANTLE?.T. SEEROOT?.T.  
 MANTSURF:10 COLOUR:lt-br NEWCOLOUR:wt-gy OLDLOUR:lt-br  
 COLOURNOTE:apices like wet etched glass RZFREQ:n EH?.F.  
 LACTICACID:shrivelled after rinsing SULFOVAN:pink root  
 LACT? .F. OMANTYPE:5 OMCELLSHAP:rounded, few jigsaw, few  
 polygonal OMSEPTAVIS?.T. OMCLAMPS?.F. OMSEPTANOT:not  
 noted OMCELLWIDE:8 µm OMCELLLEN:10 µm OMCOLOUR:none  
 OMCELLSURF:smooth OMANATNOTE:sn49 larger, mostly 16-20  
 IMANTYPE:3 IMSEPTAVIS?.T. IMCLAMPS?.F. IMSEPTANOT:not  
 noted IMCELLWIDE:4 µm IMCELLLEN:25 µm IMCOLOUR:none  
 IMCELLSURF:smooth IMANATNOTE:type towards 5 or 6, some  
 cells with granular contents (laticifers?) EHSEPTAVIS?.F.  
 EHANATNOTE:few hyphae assoc with mantle have clamps, same  
 fungus? CY?.T. CYSHAPE:straight, sharp tipped CYLEN:308  
 µm CYWIDTH:3 µm CYWALLTHIK:1 µm CYCONT:not granular  
 CYCOLOUR:none CYSURF:smooth CYANATNOTE:colour optics?,  
 shorter ones have round ends SCLEROTIA?.F.  
 MISCNOTES:maybe same as em 31=em47, but em17 has longer,  
 straighter cy

EM:18 GENUS:*Gomphidius?* SPECIES:*glutinosus?* SN:15  
 DATE:06/15/92 NTIPS:7 TTOTAL:29 CTOTAL:2 CLASS:b RAM:5  
 SYSLLEN:2 mm TIPDIAM:450  $\mu\text{m}$  TIPSHAPE:a MORPHNOTE:ram may  
 be 3, only 7 tips! SEEMANTLE?.F. SEEROOT?.F. MANTSURF:14  
 COLOUR:lt-br NEWCOLOUR:lt-br OLDLOUR:lt-br  
 COLOURNOTE:reddish tinge RZFREQ:n EH?.T. EHDENSITY:a  
 EHOCUR:all over EHLPSHAPE:mass of interwoven hyphae  
 EHMORPNOTE:same as mantle KOH:pink after rinsing LACT?  
 .F. OMATYPE:1 OMSEPTAVIS?.F. OMCELLWIDE:3  $\mu\text{m}$   
 OMCOLOUR:none OMCELLSURF:heavy encrustation of xtls  
 IMATYPE:3 IMCELLSHAP:rectangular IMSEPTAVIS?.T.  
 IMCLAMPS?.F. IMCELLWIDE:3  $\mu\text{m}$  IMCOLOUR:none  
 IMANATNOTE:type not really a 3, groups of parallel hyphae,  
 quite distinctive EHHPSHAPE:straight-gently curved  
 EHSEPTAVIS?.F. EHSEPTANOT:? EHDIAM:3  $\mu\text{m}$  EHCOLOUR:none  
 EHSURF:xtls 2X1  $\mu\text{m}$  EHRAM:60 degEHPROXIMAL:? EHDISTAL:?  
 EHJOINFREQ:? EHJOINTYPE:? CY?.F. SCLEROTIA?.F.  
 MISCNOTES:could be same as em30, only 2 occur and 21 tips,  
 could be parasitism of an ectomycorrhiza by *Gomphidius*

EM:19 SN:15 DATE:06/14/92 NTIPS:8 TTOTAL:138 CTOTAL:2  
 CLASS:a RAM:3 SYSLLEN:4 mm TIPDIAM:688  $\mu\text{m}$  AXDIAM:730  $\mu\text{m}$   
 TIPSHAPE:b MORPHNOTE:only 8 tips!, tips squeezed together,  
 after 4 days in water looks like fig16 (>30X), sn77:bit  
 like tipshape c SEEMANTLE?.T. SEEROOT?.F. MANTSURF:12  
 COLOUR:wt NEWCOLOUR:wt OLDLOUR:wt COLOURNOTE:slightly  
 pink or brown beneath, sn77: tips wh on lt-ye-br, older  
 parts gy+wh RZFREQ:n EH?.T. ETOH:cleared FESO4:n  
 KOH:cleared LACTICACID:n MELTZERS:n SULFOVAN:cleared  
 AF254:n AF366:n LACT? .F. OMATYPE:2 OMCELLSHAP:kinky  
 hyphae; round or jigsaw sometimes OMSEPTAVIS?.T.  
 OMCLAMPS?.F. OMSEPTANOT:curved OMCELLWIDE:3  $\mu\text{m}$   
 OMCELLLEN:40  $\mu\text{m}$  OMCOLOUR:none OMANATNOTE:type 5

sometimes, network of hyphae in type2, sn77: type 2 on 5  
 (size 5-10 isodiametric) (fairly thick mantle) IMANTYPE:2  
 IMSEPTAVIS?.T. IMCLAMPS?.F. IMSEPTANOT:not noted  
 IMCELLWIDE:3 µm RZANAT:none obs EHHPSHAPE:kinky, sn77:  
 wiggly EHSEPTAVIS?.F. EHSEPTANOT:? EHDIAM:2 µm  
 EHSURF:smooth EHDISTAL:rounded EHANATNOTE:sn77: 1-2 µm  
 CY?.F. SCLEROTIA?.F. MISCNOTES:fairly distinctive

EM:20 GENUS:*Amphinema* SPECIES:*byssoides* SN:16  
 DATE:07/10/92 NTIPS:16 TTOTAL:118 CTOTAL:6 CLASS:a  
 RAM:5 SYLEN:12 mm TIPDIAM:400 µm AXDIAM:440 µm  
 TIPSHAPE:b MORPHNOTE:gently curved tips SEEMANTLE?.T.  
 SEEROOT?.T. MANTSURF:11 COLOUR:br-ye NEWCOLOUR:br-ye  
 OLDLOUR:br-ye COLOURNOTE:white surface colour in places,  
 translucent in places RZFREQ:a RZOCCUR:all over  
 RZCONNECT:19 RZCOLOUR:br-ye RZDIAM:50 µm RZRAM:60  
 degrees into smaller ones RZXS:f RZSURF:23 RZNOTE:rzsurf  
 bit like 22, diam 80 µm or less, eg 20 EH?.T. EHDENSITY:a  
 EHOCCUR:sporadic HLP SHAPE:felty EHMORPNOTE:forming  
 sheets ETOH:n FESO4:extremely green root KOH:cleared and  
 darkened LACTICACID:n MELTZERS:n SULFOVAN:n AF254:n  
 AF366:n LACT?.F. OMANTYPE:1 OMSEPTAVIS?.T.  
 OMCLAMPS?.T. OMSEPTANOT:very large OMCELLWIDE:2 µm  
 OMCOLOUR:none OMCELLSURF:smooth OMANATNOTE:sn73,74: type  
 2, sn73: 3.5-4 µm, sn74: 2.5-3 µm, sn81: 4 µm,  
 sn73,74,81:regular-sized clamps IMANTYPE:3 IMSEPTAVIS?.T.  
 IMCLAMPS?.F. IMCELLWIDE:6 µm IMCELLLEN:22 µm  
 IMCOLOUR:none IMCELLSURF:smooth IMANATNOTE:length 11-33,  
 width 4.4 to 11, sn73,74: width 2,4 RZANAT:sn 73,74,81:  
 hyphae 4-7 and sometimes br-ye EHSEPTAVIS?.T.  
 EHCLAMPS?.T. EHSEPTANOT:very large clamps EHDIAM:2 µm  
 EHCOLOUR:none EHSURF:smooth EHRAM:90 deg. one obs  
 EHJOINFREQ:o EHANATNOTE:one obs of anast., old specimen,

sn74,81:some large clamps and widths 3-4 and ye CY?.F.  
 SCLEROTIA?.F. MISCNOTES:looks a but like em11,16 but is  
 yellow and the rz are a bit different

EM:21 SN:16 DATE:06/26/92 NTIPS:51 TTOTAL:139 CTOTAL:4  
 CLASS:a RAM:5 SYLEN:7 mm TIPDIAM:500  $\mu$ m AXDIAM:600  $\mu$ m  
 TIPSHAPE:c MORPHNOTE:diam 450-615, systems of intertwined  
 tips, matte not shiny (check sn29,78, etched glass)  
 SEEMANTLE?.T. SEEROOT?.T. MANTSURF:10 COLOUR:lt-or  
 NEWCOLOUR:lt-ye OLDCOLOUR:rd-or COLOURNOTE:slightly  
 textured,a few tips with purple in mantle verytip, sn29  
 check on colour RZFREQ:n EH?.T. EHDENSITY:f  
 EHOCUR:anywhere EHLPSHAPE:wispy EHMORPNOTE:forms sheet  
 in humus ETOH:none FESO4:none KOH:darkening to reddish  
 LACTICACID:none MELTZERS:none SULFOVAN:none AF254:none  
 AF366:none LACT? .F. OMANTYPE:6 OMCELLSHAP:quadrangles,  
 rectangles, triangles OMSEPTAVIS?.F. OMCELLWIDE:15  $\mu$ m  
 OMCELLEN:10  $\mu$ m OMCOLOUR:none OMCELLSURF:smooth  
 OMANATNOTE:few layers (2-3), type a bit like 5,  
 sn29:20x15,12x8,15x8,10x8,8x5,10x10 and mostly triangular,  
 sn78: similar sizes IMANTYPE:3 IMCELLSHAP:round to long  
 rectangular IMSEPTAVIS?.F. IMCELLWIDE:4  $\mu$ m IMCELLEN:15  
 $\mu$ m IMCOLOUR:br? IMCELLSURF:smooth IMANATNOTE:thick  
 walled, sn29:12x5,15x5,9x5,10x4 EHHPSHAPE:straight, gently  
 curved EHSEPTAVIS?.T. EHCLAMPS?.T. EHSEPTANOT:rounded  
 clamps EHDIAM:3  $\mu$ m EHCLOUR:none EHSURF:smooth  
 EHDISTAL:not narrowed EHANATNOTE:same fungus? sn 65b w  
 bumpy clamps CY?.F. SCLEROTIA?.F.

EM:22 SN:16 DATE:06/26/92 NTIPS:134 TTOTAL:280  
 CTOTAL:8 CLASS:a RAM:5 SYLEN:7 mm TIPDIAM:400  $\mu$ m  
 AXDIAM:650  $\mu$ m TIPSHAPE:b MORPHNOTE:some tips shape c,  
 mainly single tips or small systems of 3-6 tips, surf felty

SEEMANTLE?.T. SEEROOT?.T. MANTSURF:15 COLOUR:lt-ye  
 NEWCOLOUR:lt-ye OLDCOLOUR:rd-br RZFREQ:o RZCONNECT:20  
 RZCOLOUR:wt RZDIAM:150 µm RZXS:f RZSURF:21 EH?.T.  
 EHDENSITY:a EHOCCUR:all over EHLPSHAPE:cottony  
 EHMORPNOTE:as sheets in soil, em39: lots of mycelium in  
 soil, some as sheets ETOH:n FESO4:after rinsing dark  
 root, white mantle LACT?.F. OMATYPE:1 OMSEPTAVIS?.T.  
 OMCLAMPS?.T. OMCELLWIDE:3 µm OMCOLOUR:none  
 OMCELLSURF:smooth OMANATNOTE:some without clamps, em39:  
 very few without clamps, em39:4-6µm, sn81,83: types2,3,  
 sn83: 4 µm IMATYPE:3 IMCELLSHAP:variable IMSEPTAVIS?:F.  
 IMCELLWIDE:4 µm IMCELLLEN:14 µm IMCOLOUR:none  
 IMCELLSURF:smooth IMANATNOTE:length 11,17, width 2.7, 3.3,  
 5,6, sn78: some clamps and widths3-4 EHHPSHAPE:straight to  
 gently curved EHSEPTAVIS?.T. EHCLAMPS?.T.  
 EHSEPTANOT:rounded clamps EHDIAM:3 µm EHCOLOR:none  
 EHSURF:smooth EHRAM:60 deg. rounded EHDISTAL:not narrowed  
 EHANATNOTE:not gently widened at ramification (?), sn78:  
 thin walls and ehdiam 4, sn83: 4µm CY?.F. SCLEROTIA?.F.  
 MISCNOTES:copious eh permeates soil, making it cohesive

EM:23 SN:16 DATE:06/27/92 NTIPS:31 TTOTAL:32 CTOTAL:1  
 CLASS:c RAM:2 SYSLLEN:10 mm TIPDIAM:600 µm AXDIAM:650 µm  
 TIPSHAPE:b MORPHNOTE:tip shape towards c and d  
 SEEMANTLE?.T. SEEROOT?.T. MANTSURF:15 COLOUR:rd-br  
 NEWCOLOUR:ye-br OLDCOLOUR:rd-br COLOURNOTE:light colours  
 RZFREQ:a ZOCCUR:about junctions, midway, not at tips  
 RZCONNECT:20 RZCOLOUR:wt RZDIAM:300 µm  
 RZRAM:gradual/frequent RZXS:f RZSURF:21 RZNOTE:diam  
 range 120-650 EH?.T. EHDENSITY:a EHOCCUR:subapical  
 EHLPSHAPE:straight-gently curved ETOH:none FESO4:after  
 rinsing white with green-blue root KOH:cleared and dark  
 LACTICACID:cleared and shrivelled MELTZERS:yellowish white

after rinsing SULFOVAN:redder after rinsing AF254:none  
 AF366:none LACT? .F. OMANTYPE:1 OMCELLSHAP:like eh  
 OMSEPTAVIS?.T. OMCLAMPS?.F. OMSEPTANOT:straight thin  
 OMCELLWIDE:2 µm OMCOLOUR:none OMCELLSURF:smooth  
 IMANTYPE:3 IMCELLSHAP:long hyphae IMSEPTAVIS?.T.  
 IMCLAMPS?.F. IMSEPTANOT:straight, thin IMCELLWIDE:2 µm  
 IMCOLOUR:none IMCELLSURF:smooth IMANATNOTE:groups of  
 parallel hyphae at 90 deg to one another, frequent  
 round-about RZANAT:lots of eh, parallel hyphae  
 RZVESS:none EHHPSHAPE:straight - gently curved  
 EHSEPTAVIS?.T. EHCLAMPS?.F. EHSEPTANOT:straight thin  
 EHDIAM:2 µm EHCLOUR:none EHSURF:thin EHRAM:90deg  
 EHDISTAL:rounded, hyphae a bit wiggly EHJOINREQ:c  
 EHJOINTYPE:d1 CY?.F. SCLEROTIA?.F. MISCNOTES:non  
 distinctive, one observation

EM:24 SN:16 DATE:06/27/92 NTIPS:20 TTOTAL:22 CTOTAL:2  
 CLASS:c RAM:2 SYLEN:10 mm TIPDIAM:520 µm TIPSHAPE:b  
 MORPHNOTE:surface slightly felty in places, a bit fuzzy,  
 not shiny, hard to see, very hyaline SEEMANTLE?.T.  
 SEEROOT?.T. MANTSURF:10 COLOUR:lt-or NEWCOLOUR:lt-or  
 OLDCOLOUR:rd-br RZFREQ:n EH?.T. EHDENSITY:o  
 EHOCCUR:anywhere EHLPSHAPE:straight-gently curved  
 EHMOPNOTE:poor specimens LACTICACID:shrivelled LACT? .F.  
 OMANTYPE:1 OMSEPTAVIS?.T. OMCLAMPS?.F. OMCELLWIDE:2 µm  
 OMCOLOUR:n OMCELLSURF:smooth OMANATNOTE:thick transparent  
 mantle IMANTYPE:2 IMSEPTAVIS?.T. IMCLAMPS?.F.  
 IMCELLWIDE:2 µm IMCOLOUR:none IMCELLSURF:smooth  
 IMANATNOTE:somewhat like type 3, cell width 1.6-3.3, hyphae  
 in gelatinous matrix? EHHPSHAPE:straight-gently curved  
 EHSEPTAVIS?.T. EHCLAMPS?.F. EHDIAM:2 µm EHCLOUR:n  
 EHSURF:sm EHRAM:60 deg EHDISTAL:narrowed?  
 EHANATNOTE:poor specimen of uncertain origin CY?.F.

SCLEROTIA?.F. MISCNOTES:two observations, few tips,  
strange form

EM:25 SN:16 DATE:06/29/92 NTIPS:62 TTOTAL:78 CTOTAL:4  
CLASS:a RAM:2 SYSLEN:17 mm TIPDIAM:570  $\mu$ m AXDIAM:580  $\mu$ m  
TIPSHAPE:c MORPHNOTE:not as much of a c as the picture,  
surf finely grainy SEEMANTLE?.T. SEEROOT?.T. MANTSURF:12  
COLOUR:lt-or NEWCOLOUR:lt-or OLDCOLOUR:gy  
COLOURNOTE:reddish tones in places, sn44: or-br, sn101: old  
parts with orange patches RZFREQ:n EH?.F. ETOH:none  
FESO4:none KOH:none LACTICACID:none MELTZERS:none  
SULFOVAN:none AF254:none AF366:none LACT? .F.  
OMATYPE:4 OMCELLSHAP:jigsaw, some rounded, v. much like  
fig 4a OMSEPTAVIS?.T. OMCLAMPS?.F. OMCELLWIDE:5  $\mu$ m  
OMCELLLEN:16  $\mu$ m OMANATNOTE:brittle mantle, type 4(5) or  
5(6)(sn44), with 4 mid, sn101: type 4 IMATYPE:2  
IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:2  $\mu$ m  
IMCOLOUR:none IMCELLSURF:smooth EHHPSHAPE:wiggly  
EHSEPTAVIS?.T. EHCLAMPS?.F. EHDIAM:2  $\mu$ m EHCLOUR:none  
EHSURF:smooth EHDISTAL:rounded, not narrowed  
EHANATNOTE:few obs, width obs from sn44 CY?.F.  
SCLEROTIA?.F. MISCNOTES:like em21, but without sheets of  
mycelium, smaller and with a type 4 outer mantle more  
often, should be put with lactarius

EM:26 SN:16 DATE:06/29/92 NTIPS:8 TTOTAL:8 CTOTAL:1  
CLASS:c RAM:2 SYSLEN:6 mm TIPDIAM:550  $\mu$ m AXDIAM:780  $\mu$ m  
TIPSHAPE:b MORPHNOTE:surface frosty-sugar coated  
SEEMANTLE?.T. SEEROOT?.T. MANTSURF:11 COLOUR:wt  
NEWCOLOUR:wt OLDCOLOUR:wt COLOURNOTE:light brown beneath  
white surface texture RZFREQ:o RZOCCUR:mid tip  
RZCONNECT:19 RZCOLOUR:wt RZDIAM:100  $\mu$ m RZXS:f RZSURF:21  
EH?.F. ETOH:white gone-pale brown translucent FESO4:n

KOH:brown after rinsing LACTICACID:n MELTZERS:n  
 SULFOVAN:n AF254:n AF366:n LACT? .F. OMANTYPE:1  
 OMSEPTAVIS?.T. OMCLAMPS?.F. OMCELLWIDE:2 µm  
 OMANATNOTE:widths:2.2,2.6 IMANTYPE:3 IMSEPTAVIS?.T.  
 IMCLAMPS?.F. IMCELLWIDE:3 µm IMCOLOUR:none  
 IMCELLSURF:smooth RZANAT:parallel hyphae, 90 deg branching  
 RZVESS:none EHSEPTAVIS?.T. EHCLAMPS?.F. EHDIAM:2 µm  
 EHSURF:sm EHRAM:90 deg EHANATNOTE:widths:1.6,2.0, thin  
 walls CY?.F. SCLEROTIA?.F. MISCNOTES:very few observed

EM:27 GENUS:Amanita? SPECIES:muscaria? SN:17  
 DATE:08/06/92 NTIPS:187 TTOTAL:527 CTOTAL:14 CLASS:a  
 RAM:5 SYLEN:80 mm TIPDIAM:710 µm TIPSHAPE:b  
 MORPHNOTE:loose mantle, long sinuous tips,cortex surf a bit  
 visible SEEMANTLE?.T. SEEROOT?.F. MANTSURF:11 COLOUR:wt  
 NEWCOLOUR:wt OLDLOUR:wt RZFREQ:a RZOCCUR:all over  
 RZCONNECT:19 RZCOLOUR:wt RZDIAM:120 µm RZRAM:frequent  
 into many small strands RZXS:r RZSURF:21 EH?.T.  
 EHDENSITY:a EHOCCUR:all over ETOH:cleared mantle but not  
 rhizomorphs FES04:n KOH:cleared mantle but not  
 rhizomorphs LACTICACID:cleared and shrivelled  
 MELTZERS:dull yellow SULFOVAN:cleared mantle and  
 rhizomorphs AF254:none AF366:none LACT? .F. OMANTYPE:1  
 OMCELLSHAP:spaghetti OMSEPTAVIS?.T. OMCLAMPS?.T.  
 OMCELLWIDE:4 µm OMCOLOUR:n OMCELLSURF:lite/heavy  
 ornamented with dots/lumps OMANATNOTE:some of the dots  
 detach?, small side branches IMANTYPE:3  
 IMCELLSHAP:variable: some shorter and rounder, some long  
 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:3 µm IMCOLOUR:n  
 IMCELLSURF:sm IMANATNOTE:width 2-3 some 4 RZANAT:like  
 outer mantle EHSEPTAVIS?.F. EHANATNOTE:like outer mantle  
 CY?.F. SCLEROTIA?.F. MISCNOTES:type of ornamentation,  
 whiter, thinner mantle and small rz distinguish 27 from em2

EM:28 GENUS:"*Fagirhiza*" SPECIES:"*spinulosa*" (Agerer 1993)  
 SN:20 DATE:08/10/93 NTIPS:50 TTOTAL:151 CTOTAL:4  
 CLASS:a RAM:2 SYSLLEN:19 mm TIPDIAM:570  $\mu$ m AXDIAM:760  $\mu$ m  
 TIPSHAPE:c MORPHNOTE:tip shape not quite as much of a c as  
 in photo SEEMANTLE?.T. SEEROOT?.F. MANTSURF:16  
 COLOUR:dk-br NEWCOLOUR:br OLDCOLOUR:dk-br RZFREQ:n  
 EH?.F. KOH:darkened AF254:none AF366:n LACT?.F.  
 OMANTYPE:6 OMCELLSHAP:triangular, some quadrangles  
 OMSEPTAVIS?.F. OMCELLWIDE:14  $\mu$ m OMCELLLEN:17  $\mu$ m  
 OMCOLOUR:br OMCELLSURF:smooth OMANATNOTE:clusters of  
 setae with cells radiating outwards, eg of width 19x13,  
 11x11, 24x19, 15x11 IMANTYPE:2 IMCELLSHAP:rods with  
 uneven walls; ie bulges IMSEPTAVIS?.T. IMCLAMPS?.F.  
 IMCELLWIDE:4  $\mu$ m IMCELLLEN:32  $\mu$ m IMCOLOUR:br  
 IMCELLSURF:sm IMANATNOTE:radiating hyphae from cluster  
 centres, thin mantle EHSEPTAVIS?.F. CY?.T. CYSHAPE:flask  
 shaped CYLEN:44  $\mu$ m CYWIDTH:6  $\mu$ m CYWALLTHIK:2  $\mu$ m  
 CYCONT:none CYCOLOUR:n CYSURF:sm CYANATNOTE:in groups,  
 v. abundant, some v. short, varied base, width 2.2 at tip  
 to 9 at base, lengths 30-50, sn104: 100 $\mu$ m long, width 1 to  
 10 SCLEROTIA?.F. MISCNOTES:maybe 2 close species

EM:29 SN:29 DATE:12/21/92 NTIPS:163 TTOTAL:265  
 CTOTAL:6 CLASS:a RAM:2 SYSLLEN:14 mm TIPDIAM:610  $\mu$ m  
 AXDIAM:700  $\mu$ m TIPSHAPE:b MORPHNOTE:tips with bulbous  
 tips, velvety surface to fuzzy SEEMANTLE?.T. SEEROOT?.F.  
 MANTSURF:12 COLOUR:ye NEWCOLOUR:ye OLDCOLOUR:ye-br  
 COLOURNOTE:slightly greenish RZFREQ:o RZOCCUR:subapical  
 RZCONNECT:18 RZCOLOUR:ye-br RZDIAM:75  $\mu$ m RZXS:r  
 RZSURF:22 RZNOTE:colour probably ye when fresh EH?.F.  
 ETOH:insufficient material AF254:n AF366:n LACT?.F.  
 OMANTYPE:2 OMSEPTAVIS?.T. OMCLAMPS?.T. OMCELLWIDE:5  $\mu$ m  
 OMCOLOUR:gr-ye OMCELLSURF:sm OMANATNOTE:holds mineral

soil particles, sn20:type 2(3) IMATYPE:2 IMSEPTAVIS?.T.  
 IMCLAMPS?.T. IMSEPTANOT:parallel hyphae IMCELLEN:4 µm  
 IMCOLOUR:gr-ye IMCELLSURF:sm IMANATNOTE:parallel hyphae  
 RZANAT:ornamented eh, 3µm wide RZVESS:yes in centre (one  
 observation) VESSDIAM:10 EHSEPTAVIS?.T. EHCLAMPS?.T.  
 EHDIAM:3 µm EHCOLOUR:gr-ye EHSURF:sm EHDISTAL:rounded,  
 sometimes narrowed CY?.F. SCLEROTIA?.F.  
 MISCNOTES:distinctive

EM:30 SN:20 DATE:08/11/92 NTIPS:201 TTOTAL:201  
 CTOTAL:1 CLASS:b RAM:2 SYSLLEN:17 mm TIPDIAM:600 µm  
 AXDIAM:700 µm TIPSHAPE:c SEEMANTLE?.T. SEEROOT?.F.  
 MANTSURF:13 COLOUR:bk-wt NEWCOLOUR:bk-wt OLDLOUR:bk-wt  
 COLOURNOTE:patterns white to brown to black patches,  
 protected areas white to pinkish RZFREQ:o  
 RZOCCUR:anywhere RZCONNECT:18 RZCOLOUR:br-wt RZDIAM:200  
 µm RZXS:r RZSURF:22 RZNOTE:colour like em--patchy  
 EH?.F. ETOH:n FESO4:darker KOH:PURPLE and black  
 LACTICACID:n MELTZERS:n SULFOVAN:n AF254:n AF366:n  
 LACT? .F. OMATYPE:1 OMCELLSHAP:uneven width  
 OMSEPTAVIS?.T. OMCLAMPS?.F. OMCELLWIDE:4 µm OMCOLOUR:n  
 OMCELLSURF:ornamented OMANATNOTE:full of oily contents  
 IMATYPE:3 IMCELLSHAP:variable with hyphae IMSEPTAVIS?.T.  
 IMCLAMPS?.F. IMCELLWIDE:4 µm IMCELLSURF:sm  
 IMANATNOTE:hyphae in parallel clusters EHSEPTAVIS?.F.  
 CY?.T. CYSHAPE:round tipped with short thorn CYLEN:15 µm  
 CYWIDTH:5 µm CYCOLOUR:none CYSURF:sm CYANATNOTE:one  
 observation only, is this really a cystidium SCLEROTIA?.F.  
 MISCNOTES:could be same as em18

EM:31 SN:21 DATE:08/17/92 NTIPS:6 TTOTAL:145 CTOTAL:5  
 CLASS:a RAM:2 SYSLLEN:7 mm TIPDIAM:550 µm AXDIAM:550 µm  
 TIPSHAPE:b SEEMANTLE?.T. SEEROOT?.T. MANTSURF:17

COLOUR:or-br NEWCOLOUR:or-br OLDCOLOUR:br  
 COLOURNOTE:light orange brown, sn81:white areas due to  
 cystidia RZFREQ:n EH?.F. ETOH:n FESO4:n KOH:cleared  
 LACTICACID:n MELTZERS:n SULFOVAN:n AF254:notst  
 AF366:notst LACT? .F. OMANTYPE:6  
 OMCELLSHAP:triangular-quadrangular-polygonal  
 OMSEPTAVIS?.F. OMCELLWIDE:11  $\mu$ m OMCOLOUR:n OMCELLSURF:sm  
 OMANATNOTE:very thin, ~ one cell  
 thick,widths:11,13,8,11,14,9, sn81:2 cell layers and type  
 4-5, sn106:type 5, sn81:20x12,15x8,30x7,20x12 IMANTYPE:3  
 IMCELLSHAP:elongated, curved, uneven width IMSEPTAVIS?.T.  
 IMCLAMPS?.F. IMCELLWIDE:6  $\mu$ m IMCELLEN:22  $\mu$ m IMCOLOUR:n  
 IMCELLSURF:sm IMANATNOTE:widths:13x5,24x4,20x7,31x4, sn  
 85: 1 cell layer, sn81: 4-5  $\mu$ m EHHPSHAPE:wiggly-bumpy  
 EHSEPTAVIS?.T. EHCLAMPS?.T. EHSEPTANOT:not all septa  
 clamped EHDIAM:4  $\mu$ m EHCOLOUR:none EHSURF:sm  
 EHANATNOTE:rare. elbows. same fungus? wiggly bumpy, sn85:  
 4-8  $\mu$ m CY?.T. CYSHAPE:long.many celled.bulbous base  
 CYLEN:150  $\mu$ m CYWIDTH:5  $\mu$ m CYWALLTHIK:1  $\mu$ m CYCONT:none  
 CYCOLOUR:none CYSURF:sm CYANATNOTE:4-5 cells, tapered to  
 3.3  $\mu$ m, blunt, bases like mantle cells, sn81:(60-150(200)  
 long tho mostly 100, walls 1-2 thick, 3-6 wide)  
 SCLEROTIA?.F. MISCNOTES:could be the same as em17, same as  
 em47, see description of em47

EM:32 SN:22 DATE:08/18/92 NTIPS:62 TTOTAL:89 CTOTAL:2  
 CLASS:b RAM:2 SYSLEN:10 mm TIPDIAM:557  $\mu$ m AXDIAM:650  $\mu$ m  
 TIPSHAPE:b MORPHNOTE:slightly bent tips, some warts or  
 xtls, sn68:(few warts, few mildewy areas, mostly brown)  
 SEEMANTLE?.T. SEEROOT?.T. MANTSURF:12 COLOUR:lt-br  
 NEWCOLOUR:lt-br OLDCOLOUR:lt-br COLOURNOTE:tips and  
 apices lt-br-ye to wt, older parts whitish lt-rd-br or  
 occas. wt RZFREQ:n EH?.T. EHDENSITY:a EHOCUR:subapical

EHLPSHAPE:straight EHMORPNOTE:on some tips only, not a  
 constant feature ETOH:n FESO4:n KOH:n  
 LACTICACID:shrivelled, rehydrated when back in water  
 MELTZERS:yellowish white (rinsed in water) SULFOVAN:n  
 AF254:none AF366:none LACT? .F. OMANTYPE:5  
 OMCELLSHAP:like picture OMSEPTAVIS?.T. OMCLAMPS?.F.  
 OMCELLWIDE:7  $\mu$ m OMCELLLEN:10  $\mu$ m OMCOLOUR:n OMCELLSURF:sm  
 OMANATNOTE:type5(4), 10x8,12x8,8x5, white part 2.2-2.7,  
 sn68:(type 4, brittle) IMANTYPE:2 IMSEPTAVIS?.T.  
 IMCLAMPS?.F. IMCELLWIDE:3  $\mu$ m IMCOLOUR:n IMCELLSURF:sm  
 IMANATNOTE:difficult to see, very hyaline.sizes  
 2.2,2.2,3.3,3.3,2.2,2.2, sn68:(similar type EHSEPTAVIS?.T.  
 EHCLAMPS?.F. EHDIAM:3  $\mu$ m EHCLOUR:n EHSURF:sm  
 EHANATNOTE:sn68:(occasional wiggly eh unclamped, smooth)  
 CY?.F. SCLEROTIA?.F. MISCNOTES:close to em12

EM:33 SN:26 DATE:11/26/92 NTIPS:18 TTOTAL:18 CTOTAL:1  
 CLASS:c RAM:2 SYLEN:10 mm TIPDIAM:640  $\mu$ m AXDIAM:640  $\mu$ m  
 TIPSHAPE:c SEEMANTLE?.T. SEEROOT?.T. MANTSURF:11  
 COLOUR:ye-br NEWCOLOUR:ye-br OLDLOUR:dk-br  
 COLOURNOTE:tips lt-ye-br, apices sometimes a bit reddish  
 RZFREQ:n EH?.F. ETOH:n FESO4:n KOH:cleared  
 LACTICACID:cleared MELTZERS:n SULFOVAN:shrivelled  
 AF254:notst AF366:notst LACT? .F. OMANTYPE:3  
 OMCELLSHAP:not readily visible OMSEPTAVIS?.T.  
 OMCLAMPS?.F. OMCELLWIDE:5  $\mu$ m OMCOLOUR:n OMCELLSURF:?  
 OMANATNOTE:thin compact mantle with granules on surface  
 IMANTYPE:2 IMCELLSHAP:various, bent and slightly inflated  
 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:3  $\mu$ m IMCOLOUR:n  
 IMCELLSURF:sm IMANATNOTE:approaching type 5  
 EHSEPTAVIS?.F. CY?.F. SCLEROTIA?.F. MISCNOTES:seen once,  
 non-distinctive

EM:34 SN:29 DATE:12/21/92 NTIPS:200 TTOTAL:200  
 CTOTAL:1 CLASS:b RAM:2 SYSLLEN:9 mm TIPDIAM:670 µm  
 AXDIAM:720 µm TIPSHAPE:b MORPHNOTE:slightly  
 felty-fibrous, like tar paper, sometimes shiny  
 SEEMANTLE?.T. SEEROOT?.F. MANTSURF:11 COLOUR:dk-br  
 NEWCOLOUR:dk-br OLDLOUR:dk-br COLOURNOTE:slight coppery  
 sheen RZFREQ:n EH?.T. EHDENSITY:o EHMORPNOTE:few if any  
 ETOH:n FESO4:n KOH:darker? LACTICACID:n MELTZERS:n  
 SULFOVAN:n AF254:none AF366:none LACT? .F. OMATYPE:2  
 OMSEPTAVIS?.F. OMCELLWIDE:3 µm OMCOLOUR:dk-br  
 OMCELLSURF:sm OMANATNOTE:thick walled, dark brown, widths  
 2-3 IMATYPE:3 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:4  
 µm IMCELLLEN:15 µm IMCOLOUR:br IMCELLSURF:sm  
 IMANATNOTE:lengths13,7,15,27,15 widths3,5,2,3,5  
 EHPHSHAPE:curved EHSEPTAVIS?.F. EHDIAM:2 µm  
 EHDISTAL:narrowed CY?.T. CYSHAPE:curved to straight,  
 tapered CYLEN:85 µm CYWIDTH:3 µm CYCONT:clear  
 CYCOLOUR:br CYSURF:sm CYANATNOTE:length 60-100 µm, width  
 4 µm at base, 1.5 µm at tip, small elbows SCLEROTIA?.F.  
 MISCNOTES:bit like em38

EM:35 SN:29 DATE:12/23/92 NTIPS:22 TTOTAL:22 CTOTAL:1  
 CLASS:c RAM:1 SYSLLEN:6 mm TIPDIAM:520 µm TIPSHAPE:b  
 MORPHNOTE:thin transparent mantle, stripes of epidermal  
 cells beneath SEEMANTLE?.T. SEEROOT?.T. MANTSURF:10  
 COLOUR:lt-ye NEWCOLOUR:lt-ye OLDLOUR:lt-br RZFREQ:n  
 EH?.F. ETOH:n FESO4:n KOH:n LACTICACID:n MELTZERS:n  
 SULFOVAN:n AF254:none AF366:none LACT? .F. OMATYPE:3  
 OMSEPTAVIS?.T. OMCLAMPS?.F. OMCELLWIDE:2 µm OMCOLOUR:n  
 OMCELLSURF:sm OMANATNOTE:very thin mantle; widths 2,3,2,2  
 IMATYPE:3 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:3 µm  
 IMCOLCUR:n IMCELLSURF:sm IMANATNOTE:widths 3,3.5,3  
 EHSEPTAVIS?.F. CY?.F. SCLEROTIA?.F. MISCNOTES:one core,

non-distinctive

EM:36 SN:30 DATE:12/28/92 NTIPS:61 TTOTAL:61 CTOTAL:1  
 CLASS:b RAM:2 SYLEN:15 mm TIPDIAM:500  $\mu$ m AXDIAM:700  $\mu$ m  
 TIPSHAPE:b SEEMANTLE?.T. SEEROOT?.T. MANTSURF:10  
 COLOUR:or-br NEWCOLOUR:or-br OLDCOLOUR:br  
 COLOURNOTE:darker patches with slight reddish brown  
 RZFREQ:n EH?.F. ETOH:n FESO4:n KOH:n LACTICACID:n  
 MELTZERS:n SULFOVAN:n AF254:none AF366:none LACT? .F.  
 OMATYPE:5 OMCELLSHAP:round to triangular to polygonal  
 OMSEPTAVIS?.F. OMCELLWIDE:7  $\mu$ m OMCELLLEN:11  $\mu$ m  
 OMCOLOUR:or-br OMCELLSURF:sm OMANATNOTE:thin mantle  
 easily peeled, cell sizes 13x6,12x6,10x5,12x8 IMATYPE:2  
 IMCELLSHA?:widened at ends IMSEPTAVIS?.T. IMCLAMPS?.F.  
 IMCELLWIDE:4  $\mu$ m IMCOLOUR:n IMCELLSURF:sm  
 IMANATNOTE:widths 4,5,5,2,3,4,4 EHSEPTAVIS?.F. CY?.F.  
 SCLEROTIA?.F. MISCNOTES:only one observation, but fairly  
 distinctive

EM:37 SN:31 DATE:01/18/93 NTIPS:22 TTOTAL:22 CTOTAL:1  
 CLASS:b RAM:2 SYLEN:10 mm TIPDIAM:790  $\mu$ m AXDIAM:820  $\mu$ m  
 TIPSHAPE:b MORPHNOTE:one obs of axis width, bit of cortex  
 visible SEEMANTLE?.T. SEEROOT?.T. MANTSURF:11 COLOUR:wt  
 NEWCOLOUR:wt OLDCOLOUR:wt RZFREQ:a RZOCCUR:all over,  
 often mid tip RZCONNECT:18 RZCOLOUR:wt RZDIAM:200  $\mu$ m  
 RZRAM:30 degrees RZXS:r RZSURF:22 RZNOTE:largest 280,  
 small branch 50, compact, dense, fleshy EH?.F.  
 ETOH:cleared (not rhizomorphs) FESO4:n KOH:n  
 LACTICACID:n MELTZERS:n SULFOVAN:cleared, root orange  
 AF254:none AF366:none LACT? .F. OMATYPE:2  
 OMSEPTAVIS?.T. OMCLAMPS?.T. OMSEPTANOT:large clamps, lots  
 of clamps OMCELLWIDE:3  $\mu$ m OMCOLOUR:n OMCELLSURF:sm  
 OMANATNOTE:hyphae full of wiggly spheres, old specimen?

IMATYPE:3 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:4 µm  
 IMCOLOUR:n IMCELLSURF:sm RZANAT:central hyphae full of  
 wiggly spheres RZVESS:none VESSDIAM:5 EHSEPTAVIS?.F.  
 CY?.F. SCLEROTIA?.F. MISCNOTES:one core

EM:38 SN:31 DATE:01/18/93 NTIPS:19 TTOTAL:19 CTOTAL:1  
 CLASS:b RAM:2 SYLEN:10 mm TIPDIAM:440 µm AXDIAM:550 µm  
 TIPSHAPE:c MORPHNOTE:shorter tips are shape b  
 SEEMANTLE?.T. SEEROOT?.T. MANTSURF:10 COLOUR:bk-br  
 NEWCOLOUR:gr-br OLDCOLOUR:bk COLOURNOTE:some tips bk,  
 apices slightly greenish brown, dull RZFREQ:n EH?.F.  
 ETOH:insufficient material AF254:none AF366:none LACT?  
 .F. OMATYPE:2 OMSEPTAVIS?.T. OMCLAMPS?.F. OMCELLWIDE:3  
 µm OMCOLOUR:n OMCELLSURF:sm OMANATNOTE:hard to see cells  
 or hyphae, dark hyphae? IMATYPE:3 IMCELLSHAP:almost  
 epidermoid, but a bit variable IMSEPTAVIS?.T.  
 IMCLAMPS?.F. IMCELLWIDE:3 µm  
 IMANATNOTE:widths:4,3,4,5,5,2.5,2,3,4,4 EHHPSHAPE:wiggly  
 EHSEPTAVIS?.T. EHCLAMPS?.F. EHDIAM:2 µm EHWALLTHIK:1 µm  
 EHCOLOUR:br EHSURF:sm EHANATNOTE:swellings or round cells  
 CY?.F. SCLEROTIA?.F. MISCNOTES:bit like em34, one  
 observation

EM:39 SN:34 DATE:01/23/93 NTIPS:109 TTOTAL:153  
 CTOTAL:3 CLASS:b RAM:5 SYLEN:12 mm TIPDIAM:450 µm  
 AXDIAM:525 µm TIPSHAPE:c MORPHNOTE:some tips like b or d,  
 surface sugar-coated, sn49: mantsurf 11 SEEMANTLE?.T.  
 SEEROOT?.T. MANTSURF:12 COLOUR:wt NEWCOLOUR:wt  
 OLDCOLOUR:wt COLOURNOTE:some tips staining or bruising  
 yellowish brown, sn65:no staining RZFREQ:a RZOCUR:all  
 over, sn49: some on side parallel to root RZCONNECT:19  
 RZCOLOUR:wt RZDIAM:50 µm RZXS:r RZSURF:23 RZNOTE:not  
 quite as hairy as 23 EH?.T. EHDENSITY:c EHOCUR:all over

EHLPSHAPE:wispy, straight to gentle curve ETOH:almost  
 cleared FESO4:black cortex KOH:cleared and darkened  
 LACTICACID:shrivelled MELTZERS:n SULFOVAN:n AF254:none  
 AF366:none LACT? .F. OMATYPE:2 OMCELLSHAP:sn49: some  
 enlarged swollen sections OMSEPTAVIS?.T. OMCLAMPS?.F.  
 OMCOLOUR:n OMCELLSURF:sm, sn49:some large ye with large  
 bumps OMANATNOTE:many strongly refractive bodies, sn49:  
 loose mantle IMATYPE:3 IMSEPTAVIS?.T. IMCLAMPS?.F.  
 IMCELLWIDE:3  $\mu\text{m}$  IMCOLOUR:n IMCELLSURF:sm IMANATNOTE:some  
 cells widened to 6  $\mu\text{m}$ , sizes:3,2.5,2,3 RZANAT:some clamps  
 EHLPSHAPE:straight to sn 49: gently curved to wiggly  
 EHSEPTAVIS?.T. EHCLAMPS?.T. EHSEPTANOT:some clamps sn49  
 EHDIAM:3  $\mu\text{m}$  EHCOLOR:n EHSURF:smooth, some with globules  
 EHDISTAL:rounder EHANATNOTE:sn49: some 4-5 $\mu\text{m}$ . some yellow.  
 a few large (6 $\mu\text{m}$ ) yellow with large lumps CY?.F.  
 SCLEROTIA?.F. MISCNOTES:coarser ornamentation and less  
 consistent clamping than em27, 3 obs, but variable, may be  
 several fungi

EM:40 SN:38 DATE:02/12/93 NTIPS:5 TTOTAL:6 CTOTAL:1  
 CLASS:b RAM:2 SYSLLEN:6 mm TIPDIAM:531  $\mu\text{m}$  AXDIAM:585  $\mu\text{m}$   
 TIPSHAPE:b SEEMANTLE?.T. SEEROOT?.T. MANTSURF:17  
 COLOUR:ye-br NEWCOLOUR:ye-br OLDLOUR:gy-br RZFREQ:n  
 EH?.F. ETOH:not tested FESO4:not tested KOH:not tested  
 LACTICACID:not tested MELTZERS:not tested SULFOVAN:not  
 tested AF254:none AF366:none LACT? .F. OMATYPE:6  
 OMCELLSHAP:roundish to triangular OMSEPTAVIS?.F.  
 OMCELLWIDE:7  $\mu\text{m}$  OMCELLLEN:13  $\mu\text{m}$  OMCOLOUR:n OMCELLSURF:sm  
 OMANATNOTE:very thin mantle, type 6(5) 11x7,15x9,20x6,10x6  
 IMATYPE:3 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCOLOUR:n  
 IMCELLSURF:sm IMANATNOTE:could be type 6 hard to tell  
 because of thinness of mantle EHSEPTAVIS?.F. CY?.T.  
 CYSHAPE:straight CYLEN:300  $\mu\text{m}$  CYWIDTH:4  $\mu\text{m}$  CYWALLTHIK:1

µm CYCONT:refractive CYCOLOUR:n CYSURF:sm  
 CYANATNOTE:slightly narrower at tip, single celled  
 SCLEROTIA?.F. MISCNOTES:like em 17 and 31, but with  
 thicker walls in outer mantle and cystidia

EM:41 GENUS:"*Piceirhiza*" SPECIES:"*bicolorata*" SN:39  
 DATE:02/16/93 NTIPS:19 TTOTAL:19 CTOTAL:1 CLASS:b  
 RAM:1 SYSLEN:4 mm TIPDIAM:430 µm TIPSHAPE:b  
 MORPHNOTE:surface of cortex visible at tips SEEMANTLE?.T.  
 SEEROOT?.F. MANTSURF:10 COLOUR:dk-br NEWCOLOUR:br  
 OLDLOUR:dk-br COLOURNOTE:some tips bk RZFREQ:n EH?.F.  
 ETOH:none FESO4:none KOH:none LACTICACID:none  
 MELTZERS:none SULFOVAN:none AF254:none AF366:none LACT?  
 .F. OMATYPE:2 OMCELLSHAP:wiggly, bony bends  
 OMSEPTAVIS?.T. OMCLAMPS?.F. OMCELLWIDE:2 µm OMCOLOUR:br  
 OMCELLSURF:sm OMANATNOTE:widths 2,1.5,2,2 IMATYPE:3  
 IMCELLSHAP:brain pattern IMSEPTAVIS?.F. IMCELLWIDE:2 µm  
 IMCOLOUR:br IMCELLSURF:sm IMANATNOTE:brain pattern,  
 thick-walled cells EHSEPTAVIS?.F. CY?.F. SCLEROTIA?.F.  
 MISCNOTES:one obs, otherwise distinctive

EM:42 SN:42 DATE:03/08/93 NTIPS:6 TTOTAL:24 CTOTAL:2  
 CLASS:a RAM:2 SYSLEN:6 mm TIPDIAM:480 µm AXDIAM:710 µm  
 TIPSHAPE:c MORPHNOTE:fuzzy surface SEEMANTLE?.T.  
 SEEROOT?.T. MANTSURF:12 COLOUR:br NEWCOLOUR:lt-br  
 OLDLOUR:dk-br RZFREQ:n EH?.T. EHDENSITY:a EHOCCUR:all  
 over EHLPSHAPE:wiggly EHMORPNOTE:give fuzzy appearance  
 FESO4:not tested chemicals AF254:none AF366:none LACT?  
 .F. OMATYPE:5 OMCELLSHAP:various OMSEPTAVIS?.F.  
 OMCELLWIDE:21 µm OMCELLLEN:37 µm OMCOLOUR:n  
 OMCELLSURF:sm OMANATNOTE:thick-walled (2.5-3 µm),  
 35x30,30x28,30x12,20x15 sn82:(15-20 wide and same thick  
 walls, same type) IMATYPE:3 IMSEPTAVIS?.T. IMCLAMPS?.F.

IMCELLWIDE:8 µm IMCOLOUR:n IMCELLSURF:sm  
 IMANATNOTE:hartig net present, sn82: width 7-10  
 EHHPSHAPE:wiggly a bit, some spiral EHSEPTAVIS?.T.  
 EHCLAMPS?.F. EHDIAM:8 µm EHWALLTHIK:2 µm EHCOLOUR:n  
 EHSURF:sm CY?.T. CYSHAPE:spurs or teeth, sharp ,sn82:  
 varied CYLEN:10 µm CYWIDTH:6 µm CYWALLTHIK:1 µm  
 CYCOLOUR:n CYSURF:sm CYANATNOTE:length 5-15, width 3-10,  
 sn82:(sizes 10x50, 25x25 round with tip, 40x20, walls 1-3  
 µm) SCLEROTIA?.F. MISCNOTES:distinctive!!

EM:43 SN:44 DATE:03/09/93 NTIPS:59 TTOTAL:745 CTOTAL:5  
 CLASS:a RAM:2 SYSLLEN:10 mm TIPDIAM:590 µm AXDIAM:653 µm  
 TIPSHAPE:a MORPHNOTE:some tips shape b, surface shiny  
 SEEMANTLE?.T. SEEROOT?.T. MANTSURF:10 COLOUR:br-ye  
 NEWCOLOUR:br-ye OLDLOUR:br COLOURNOTE:some tips lt-br  
 RZFREQ:n EH?.T. EHDENSITY:a EHOCUR:cottony mass all  
 over EHLPSHAPE:cottony ETOH:n FESO4:root darkened,  
 mantle appears thicker KOH:cleared and darkened  
 LACTICACID:shrivelled MELTZERS:n SULFOVAN:pinkish  
 AF254:none AF366:none LACT? .F. OMANTYPE:4  
 OMCELLSHAP:mantle 1 cell-layer thick ??? OMSEPTAVIS?.T.  
 OMCLAMPS?.T. OMCOLOUR:n OMCELLSURF:sm OMANATNOTE:gelly,  
 thin transparent, hard to see cells, sn106:(some cells  
 visible: type 2(3-4), some) IMANTYPE:4  
 IMCELLSHAP:epidermoid IMSEPTAVIS?.F. IMCELLWIDE:3 µm  
 IMCELLLEN:12 µm IMCOLOUR:n IMCELLSURF:sm  
 IMANATNOTE:12x2,14x3,12x5,10x3,10x2 EHHPSHAPE:gently  
 curved, copious cottony EHSEPTAVIS?.T. EHCLAMPS?.T.  
 EHSEPTANOT:bulbous clamps EHDIAM:5 µm EHCOLOUR:n  
 EHSURF:round bumps to 1 µm diam EHRAM:90 degrees  
 EHDISTAL:rounded EHANATNOTE:clamps hemispherical or  
 larger, sn106:walls 1 µm, sn94:diam 3 w fine orn. CY?.F.  
 SCLEROTIA?.F. MISCNOTES:hyphae very similar to em39 and

together in sn49, same???

EM:44 GENUS:Tuber SN:47 DATE:03/16/93 NTIPS:34  
 TTOTAL:34 CTOTAL:1 CLASS:b RAM:2 SYSLEN:15 mm  
 TIPDIAM:700  $\mu\text{m}$  AXDIAM:950  $\mu\text{m}$  TIPSHAPE:b MORPHNOTE:some  
 tips a shaped, tips taper to 530 sometimes, surface  
 sometimes 17 texture SEEMANTLE?.T. SEEROOT?.F.  
 MANTSURF:16 COLOUR:br NEWCOLOUR:br OLDLOUR:dk-br  
 COLOURNOTE:tips and apices lt-br to dk-br, old tips can be  
 bk RZFREQ:n EH?.F. ETOH:none FESO4:none KOH:none  
 LACTICACID:highly shrivelled MELTZERS:none SULFOVAN:none  
 AF254:none AF366:none LACT? .F. OMANTYPE:5  
 OMCELLSHAP:oval to polygonal to circular OMSEPTAVIS?.F.  
 OMCELLWIDE:9  $\mu\text{m}$  OMCELLLEN:12  $\mu\text{m}$  OMCOLOUR:br  
 OMCELLSURF:sm  
 OMANATNOTE:20x10,6x9,11x9,13r,10x7,10x11,very thick mantle  
 IMANTYPE:3 IMCELLSHAP:bent, some round but in xs?  
 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:5  $\mu\text{m}$  IMCELLLEN:13  
 $\mu\text{m}$  IMCOLOUR:br IMCELLSURF:sm IMANATNOTE:radiating  
 patterns,lengths 25,10,13, widths 4,3,6,4,9,4  
 EHSEPTAVIS?.F. CY?.T. CYSHAPE:bit wiggly, gently curved  
 to straight CYLEN:100  $\mu\text{m}$  CYWIDTH:7  $\mu\text{m}$  CYWALLTHIK:1  $\mu\text{m}$   
 CYCONT:refractive CYCOLOUR:br CYSURF:sm CYANATNOTE:1 or  
 2-celled, short basal cell, base occas bulbous  
 SCLEROTIA?.F. MISCNOTES:very similar to em28, one obs

EM:45 SN:49 DATE:05/17/93 NTIPS:102 TTOTAL:102  
 CTOTAL:1 CLASS:b RAM:2 SYSLEN:22 mm TIPDIAM:590  $\mu\text{m}$   
 AXDIAM:730  $\mu\text{m}$  TIPSHAPE:b MORPHNOTE:finely fuzzy  
 SEEMANTLE?.T. SEEROOT?.F. MANTSURF:11 COLOUR:dk-br  
 NEWCOLOUR:dk-br OLDLOUR:dk-br RZFREQ:n EH?.F.  
 ETOH:none FESO4:none KOH:none LACTICACID:none  
 MELTZERS:none SULFOVAN:none LACT? .F. OMANTYPE:2

OMCELLSHAP:ham-bone, convoluted wiggly OMSEPTAVIS?.T.  
 OMCLAMPS?.T. OMCELLWIDE:5  $\mu$ m OMCOLOUR:br  
 OMCELLSURF:finely ornamented OMANATNOTE:width 4-8:  
 6,7,5,4,5 IMANTYPE:3 IMCELLSHAP:various IMSEPTAVIS?.T.  
 IMCLAMPS?.F. IMCELLWIDE:4  $\mu$ m IMCOLOUR:br  
 IMCELLSURF:smooth IMANATNOTE:some star patterning, width  
 3-4, type 3-4 EHSEPTAVIS?.F. CY?.T. CYSHAPE:triangular,  
 awl, sharp CYLEN:22  $\mu$ m CYWIDTH:7  $\mu$ m CYWALLTHIK:1  $\mu$ m  
 CYCONT:none CYCOLOUR:br CYSURF:smooth  
 CYANATNOTE:16x8(base), 22x10, 24x7, 27x5 SCLEROTIA?.F.  
 MISCNOTES:this may be em9, very similar except has cystidia

EM:46 SN:49 DATE:05/18/93 NTIPS:38 TTOTAL:74 CTOTAL:6  
 CLASS:a RAM:2 SYLEN:6 mm TIPDIAM:460  $\mu$ m TIPSHAPE:  
 MORPHNOTE:sn70-loose mantle, surface of cortex visible in  
 places SEEMANTLE?.T. SEEROOT?.F. MANTSURF:11  
 COLOUR:pk-wt NEWCOLOUR:pk-wt OLDLOUR:br  
 COLOURNOTE:reddish-brown-pink-white RZFREQ:a RZOCCUR:all  
 over RZCONNECT:19 RZCOLOUR:pk-wt RZDIAM:140  $\mu$ m RZXS:r  
 RZSURF:23 RZNOTE:rzdiam<140 EH?.T. EHDENSITY:a  
 EHOCUR:all over EHLPSHAPE:very finely fuzzy ETOH:n  
 FES04:n KOH:blue rhizomorphs! LACTICACID:n  
 MELTZERS:mantle whitish SULFOVAN:n AF254:ye AF366:bt-ye  
 LACT?.F. OMANTYPE:2 OMSEPTAVIS?.T. OMCLAMPS?.T.  
 OMSEPTANOT:constriction often OMCELLWIDE:4  $\mu$ m OMCOLOUR:n  
 OMCELLSURF:sm OMANATNOTE:sn70-type1, sn70-width3-4, width  
 4,4,5,4.5,5,4.5,4,5, sn77:(finely orn. 4.5  $\mu$ m) IMANTYPE:3  
 IMCELLSHAP:various IMSEPTAVIS?.T. IMCLAMPS?.F.  
 RZANAT:some hyphae heavily orn fine dots, 4-5 $\mu$ m, pk-or,  
 sn70,77:same EHHPSHAPE:gently curved EHSEPTAVIS?.T.  
 EHCLAMPS?.T. EHSEPTANOT:constricted EHDIAM:4  $\mu$ m  
 EHCOLOUR:n EHSURF:sm EHRAM:infrequent CY?.F.  
 SCLEROTIA?.F. MISCNOTES:sn49-9tips, sn61-25tips,

sn70-4tips, very distinctive , consistently fluorescent

EM:47 SN:51 DATE:05/22/93 NTIPS:28 TTOTAL:0 CTOTAL:0  
 CLASS: RAM:2 SYSLIN:10 mm TIPDIAM:820 µm TIPSHAPE:b  
 MORPHNOTE:some air held causes white bits, frosting  
 appearance SEEMANTLE?.T. SEEROOT?.T. MANTSURF:11  
 COLOUR:lt-br NEWCOLOUR:lt-ye OLDCOLOUR:br RZFREQ:n  
 EH?.F. ETOH:none FESO4:none KOH:none LACTICACID:none  
 MELTZERS:none SULFOVAN:none AF254:none AF366:none LACT?  
 .F. OMANTYPE:5 OMCELLSHAP:round to rectangular to  
 polygonal to irregular OMSEPTAVIS?.F. OMCELLWIDE:10 µm  
 OMCELLLEN:18 µm OMCOLOUR:n OMCELLSURF:sm OMANATNOTE:very  
 thin mantle--1-2 layers thick, type 4-5 IMANTYPE:3  
 IMCELLSHAP:various IMSEPTAVIS?.T. IMCLAMPS?.F.  
 IMCELLWIDE:6 µm IMCELLLEN:11 µm IMCOLOUR:n IMCELLSURF:sm  
 IMANATNOTE:hard to see, not really a layer, type 3-5  
 EHSEPTAVIS?.F. CY?.T. CYSHAPE:gently curved, round tipped  
 CYLEN:80 µm CYWIDTH:5 µm CYWALLTHIK:1 µm CYCONT:none  
 CYCOLOUR:none CYSURF:smooth CYANATNOTE:occasionally  
 singly septate, some clamps, basal cell like mantle cells,  
 32-180 long, 3.5-6.5 wide SCLEROTIA?.F. MISCNOTES:same as  
 em31, see description of em31

EM:48 SN:51 DATE:05/24/93 NTIPS:45 TTOTAL:45 CTOTAL:1  
 CLASS:b RAM:2 SYSLIN:10 mm TIPDIAM:700 µm AXDIAM:720 µm  
 TIPSHAPE:b MORPHNOTE:surface 11 or 14 (reticulate or  
 cottony) SEEMANTLE?.T. SEEROOT?.T. MANTSURF:11  
 COLOUR:lt-ye NEWCOLOUR:lt-ye OLDCOLOUR:lt-br RZFREQ:n  
 EH?.T. EHDENSITY:a EHOCCUR:all over EHLPSHAPE:wispy in  
 cottony mass EHMORPHNOTE:especially on older parts  
 ETOH:none FESO4:none KOH:none LACTICACID:none  
 MELTZERS:none SULFOVAN:none AF254:none AF366:none LACT?  
 .F. OMANTYPE:2 OMSEPTAVIS?.T. OMCLAMPS?.F. OMCELLWIDE:3

$\mu\text{m}$  OMCOLOUR:n OMCELLSURF:sm OMANATNOTE:very wiggly  
 hyphae, 2.5-3  $\mu\text{m}$  wide IMANTYPE:3 IMSEPTAVIS?.T.  
 IMCLAMPS?.F. IMCELLWIDE:3  $\mu\text{m}$  IMCOLOUR:n IMCELLSURF:sm  
 EHSEPTAVIS?.T. EHCLAMPS?.F. EHDIAM:1  $\mu\text{m}$  EHCOLOUR:n  
 EHSURF:sm EHRAM:with triangular junction  
 EHPROXIMAL:wider, eg 2-3 EHDISTAL:very thin CY?.T.  
 CYSHAPE:spear CYLEN:19  $\mu\text{m}$  CYANATNOTE:widths 25,30,10,10  
 SCLEPOTIA?.F. MISCNOTES:similar to em64, one observation

EM:49 SN:59 DATE:06/02/93 NTIPS:10 TTOTAL:26 CTOTAL:2  
 CLASS:c RAM:2 SYLEN:6 mm TIPDIAM:640  $\mu\text{m}$  TIPSHAPE:b  
 MORPHNOTE:ram could be 5, mantsurf like old crusty snow  
 SEEMANTLE?.T. SEEROOT?.F. MANTSURF:11 COLOUR:wt  
 NEWCOLOUR:wt OLDLOUR:wt RZFREQ:a RZOCCUR:all over  
 RZCONNECT:19 RZCOLOUR:wt RZDIAM:40  $\mu\text{m}$  RZXS:f RZSURF:23  
 RZNOTE:rzxs f?, diam 30-40 EH?.T. EHDENSITY:o ETOH:none  
 FESO4:none KOH:none LACTICACID:none MELTZERS:none  
 SULFOVAN:none AF254:none AF366:none LACT? .F.  
 OMANTYPE:1 OMSEPTAVIS?.T. OMCLAMPS?.F. OMCELLWIDE:3  $\mu\text{m}$   
 OMCOLOUR:n OMCELLSURF:finely ornamented IMANTYPE:3  
 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:4  $\mu\text{m}$  IMCOLOUR:n  
 IMCELLSURF:finely ornamented IMANATNOTE:parallel hyphae  
 RZANAT:dense, few eh, hyphae orn, diam 40,30,20,35,10  
 EHHPSHAPE:like outer mantle EHSEPTAVIS?.T. EHCLAMPS?.F.  
 EHANATNOTE:like outer mantle CY?.F. SCLEROTIA?.F.  
 MISCNOTES:one observation, non-distinctive

EM:50 SN:67 DATE:06/15/93 NTIPS:9 TTOTAL:80 CTOTAL:2  
 CLASS:b RAM:2 SYLEN:4 mm TIPDIAM:658  $\mu\text{m}$  AXDIAM:635  $\mu\text{m}$   
 TIPSHAPE:c MORPHNOTE:surface smooth to mat to warty  
 SEEMANTLE?.T. SEEROOT?.F. MANTSURF:11 COLOUR:lt-br  
 NEWCOLOUR:lt-br OLDLOUR:gy RZFREQ:n EH?.T.  
 EHDENSITY:c EHMORPNOTE:not noticeable at 40x ETOH:none

FESO4:none KOH:none LACTICACID:none MELTZERS:none  
 SULFOVAN:none AF254:none AF366:none LACT? .F.  
 OMANTYPE:5 OMCELLSHAP:roundish OMSEPTAVIS?.T.  
 OMCLAMPS?.F. OMCELLWIDE:9 µm OMCELLEN:14 µm OMCOLOUR:n  
 OMCELLSURF:smooth OMANATNOTE:type 5 with type 2 on top in  
 places IMANTYPE:3 IMSEPTAVIS?.T. IMCLAMPS?.F.  
 IMCELLWIDE:3 µm IMCOLOUR:none IMCELLSURF:smooth  
 EHHPSHAPE:wiggly EHSEPTAVIS?.T. EHCLAMPS?.F.  
 EHSEPTANOT:curved EHDIAM:3 µm EHCOLOUR:n EHSURF:sm  
 CY?.F. SCLEROTIA?.F. MISCNOTES:like em12 and em32, could  
 be em12, but 50's tips are bent

EM:51 SN:68 DATE:06/25/93 NTIPS:45 TTOTAL:45 CTOTAL:1  
 CLASS:b RAM:2 SYSLEN:9 mm TIPDIAM:724 µm AXDIAM:776 µm  
 TIPSHAPE:a MORPHNOTE:newer parts smooth, shiny; slightly  
 felty and whitish older parts SEEMANTLE?.T. SEEROOT?.T.  
 MANTSURF:10 COLOUR:lt-br NEWCOLOUR:lt-ye OLDCOLOUR:lt-br  
 COLOURNOTE:many dark stripes of epidermal cells, hyaline  
 mantle RZFREQ:n EH?.T. EHDENSITY:c EHOCCUR:variable  
 EHMORPNOTE:not on all tips ETOH:none FESO4:none KOH:none  
 LACTICACID:none MELTZERS:none SULFOVAN:none AF254:none  
 AF366:none LACT? .F. OMANTYPE:2 OMSEPTAVIS?.T.  
 OMCLAMPS?.F. OMCELLWIDE:3 µm OMCOLOUR:n OMCELLSURF:sm  
 OMANATNOTE:widths 3,4,2.5,3.5,2.5,2.5 IMANTYPE:3  
 IMCELLSHAP:like ingleby's picture IMSEPTAVIS?.T.  
 IMCLAMPS?.F. IMCELLWIDE:2 µm IMCELLEN:14 µm IMCOLOUR:n  
 IMCELLSURF:sm EHHPSHAPE:fairly straight, few obs  
 EHSEPTAVIS?.T. EHCLAMPS?.F. EHDIAM:2 µm EHCOLOUR:n  
 EHSURF:sm EHANATNOTE:widths 2,2,2 CY?.F. SCLEROTIA?.F.  
 MISCNOTES:one observation, like em6

EM:52 SN:70 DATE:07/16/93 NTIPS:22 TTOTAL:36 CTOTAL:2  
 CLASS:b RAM:2 SYSLEN:12 mm TIPDIAM:550 µm AXDIAM:730 µm

TIPSHAPE:b MORPHNOTE:tipshape towards c, SEEMANTLE?.T.  
 SEEROOT?.F. MANTSURF:12 COLOUR:wt NEWCOLOUR:wt  
 OLDCOLOUR:wt COLOURNOTE:older parts a bit yellowish  
 RZFREQ:c RZOCCUR:anywhere RZCONNECT:18 RZCOLOUR:wt  
 RZDIAM:60  $\mu$ m RZRAM:frequent 30-60 deg RZXS:r RZSURF:22  
 RZNOTE:surface roughened by xtls EH?.F. AF254:none  
 AF366:none LACT? .F. OMANTYPE:1 OMSEPTAVIS?.T.  
 OMCLAMPS?.F. OMCELLWIDE:2  $\mu$ m OMCOLOUR:gy OMCELLSURF:sm  
 OMANATNOTE:hyphae obscured by translucent xtl, gy  
 refractive, sn86:(some to 8 $\mu$ m very thin walled, others 2-3  
 finely ornamented) IMANTYPE:3 IMSEPTAVIS?.T.  
 IMCLAMPS?.F. IMCELLWIDE:4  $\mu$ m IMCOLOUR:n IMCELLSURF:sm  
 IMANATNOTE:width 3.5(6) RZANAT:fleshy, wide angle  
 branches, woven, 1.5  $\mu$ m clampless EHHPSHAPE:rare, most  
 1.5 $\mu$ m, some 2.3 $\mu$ m EHSEPTAVIS?.T. EHCLAMPS?.F. EHDIAM:2  
 $\mu$ m EHCOLOR:n EHSURF:sm EHANATNOTE:like outer mantle and  
 rz CY?.F. SCLEROTIA?.F. MISCNOTES:39 stains, has bigger  
 inner hypha; 26 has rhizomorphs with parallel hyphae, no  
 crystals; 2 observations, variable

EM:54 SN:74 DATE:12/13/93 NTIPS:7 TTOTAL:7 CTOTAL:1  
 CLASS:b RAM:2 SYLEN:2 mm TIPDIAM:435  $\mu$ m AXDIAM:470  $\mu$ m  
 TIPSHAPE:a MORPHNOTE:tip shape maybe b, short tips  
 SEEMANTLE?.T. SEEROOT?.F. MANTSURF:12 COLOUR:wh  
 NEWCOLOUR:wh OLDCOLOUR:wh COLOURNOTE:few obs, wee bits of  
 cortex visible thru mantle, sugar coated to finely fuzzy  
 RZFREQ:a RZOCCUR:at base of tips RZCONNECT:18  
 RZCOLOUR:wh RZDIAM:60  $\mu$ m RZRAM:60 RZXS:r RZSURF:22  
 RZNOTE:surface 22-23, slightly hairy with cystidia EH?.F.  
 ETOH:insufficient material AF254:none AF366:none LACT?  
 .F. OMANTYPE:2 OMCELLSHAP:rectangular OMSEPTAVIS?.T.  
 OMCLAMPS?.F. OMCELLWIDE:7  $\mu$ m OMCELLLEN:30  $\mu$ m OMCOLOUR:n  
 OMCELLSURF:many with globular deposits OMANATNOTE:some

walls 1µm, granular contents, 4.5-8 x 20-35 IMANTYPE:2  
 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:3 µm IMCOLOUR:n  
 IMCELLSURF:sm IMANATNOTE:many groups of a few parallel  
 hyphae RZANAT:parallel orn. hyphae wo spaces between,  
 gran. cont RZVESS:few, w swellings, w cystidia,  
 VESSDIAM:12 EHSEPTAVIS?.F. CY?.T. CYSHAPE:club shaped  
 CYLEN:90 µm CYWIDTH:6 µm CYWALLTHIK:1 µm CYCOLOUR:n  
 CYSURF:deposits except on distal cell CYANATNOTE:3-4  
 celled with larger basal cell and bulbous tip  
 SCLEROTIA?.F. MISCNOTES:like 39,52 but no staining, with  
 cystidia, and larger hyphae, distinctive, but few tips and  
 one observation

EM:55 SN:74 DATE:12/13/93 NTIPS:14 TTOTAL:24 CTOTAL:2  
 CLASS:b RAM:2 SYLEN:12 mm TIPDIAM:340 µm TIPSHAPE:a  
 SEEMANTLE?.T. SEEROOT?.F. MANTSURF:13 COLOUR:wh  
 NEWCOLOUR:wh OLDLOUR: COLOURNOTE:loose mantle lots of  
 brown showing thru in places RZFREQ:a RZOCUR:all over  
 RZCONNECT:20 RZCOLOUR:wh RZDIAM:80 µm RZXS:r RZSURF:21  
 RZNOTE:to 140 wide EH?.T. EHDENSITY:a EHOCUR:all over  
 EHLPSHAPE:woolly ETOH:not FESO4:not KOH:not  
 LACTICACID:not MELTZERS:not SULFOVAN:not AF254:not  
 AF366:not LACT? .F. OMANTYPE:1 OMCELLSHAP:smoothly  
 curved OMSEPTAVIS?.T. OMCLAMPS?.F. OMCELLWIDE:4 µm  
 OMCOLOUR:n OMCELLSURF:sm OMANATNOTE:very loose mantle,  
 doesn't come off in piece IMANTYPE:2 IMCELLSHAP:network  
 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:3 µm IMCOLOUR:n  
 IMCELLSURF:sm IMANATNOTE:2.5-3.5 wide, few obs  
 RZANAT:ornamented hyphae close, parallel w H-anastomoses  
 EHHPSHAPE:straight to gently curved EHSEPTAVIS?.T.  
 EHCLAMPS?.F. EHSEPTANOT:frequent septation EHDIAM:4 µm  
 EHCOLOUR:n EHSURF:finely orn. to rough, some sm EHRAM:90  
 deg, infrequent EHDISTAL:rounded EHJOINFREQ:o

EHANATNOTE:frequent small bumps CY?.F. SCLEROTIA?.F.  
 MISCNOTES:distinctive, but with few tips and only 2  
 observations

EM:56 SN:73 DATE:12/07/93 NTIPS:36 TTOTAL:115 CTOTAL:4  
 CLASS:b RAM:2 SYSLLEN:5 mm TIPDIAM:530  $\mu$ m AXDIAM:550  $\mu$ m  
 TIPSHAPE:b MORPHNOTE:1 obs of axdiam, many v. short side  
 branches/protuberances SEEMANTLE?.T. SEEROOT?.T.  
 MANTSURF:10 COLOUR:ye-br NEWCOLOUR:ye-br OLDLOUR:wh-br  
 COLOURNOTE:apices sometimes grey when actively growing  
 RZFREQ:n EH?.F. ETOH:not FESO4:not KOH:not  
 LACTICACID:not MELTZERS:not SULFOVAN:not AF254:not  
 AF366:not LACT? .F. OMANTYPE:6 OMCELLSHAP:triangular to  
 polygonal OMSEPTAVIS?.F. OMCELLWIDE:15  $\mu$ m OMCOLOUR:n  
 OMCELLSURF:sm OMANATNOTE:sn81:( mostly round cells to 18 $\mu$ m  
 wide (type 5)), sn74and109:to 18 $\mu$ m IMANTYPE:3  
 IMCELLSHAP:much like fig 2.3 IMSEPTAVIS?.T. IMCLAMPS?.F.  
 IMCELLWIDE:2  $\mu$ m IMCELLLEN:12  $\mu$ m IMCOLOUR:n IMCELLSURF:sm  
 IMANATNOTE:lengths: 12,7,15,2,30. sn74:(widths 3,2,4, type4  
 in places) sn81:type3 EHHPSHAPE:wiggly, few none in sn81,  
 sn109:(wiggly, few) EHSEPTAVIS?.T. EHCLAMPS?.F. EHDIAM:2  
 $\mu$ m EHCOLOUR:n EHSURF:sm EHANATNOTE:widths 1,2,1,  
 sn109:(no clamps, diam 1,2) CY?.F. SCLEROTIA?.F.  
 MISCNOTES:not 36 because not orange, not 50 because no  
 warts, variable, maybe more than one fungus

EM:57 GENUS:*Lactarius* SN:75 DATE:12/22/93 NTIPS:72  
 TTOTAL:225 CTOTAL:4 CLASS:a RAM:2 SYSLLEN:3 mm  
 TIPDIAM:400  $\mu$ m TIPSHAPE:b SEEMANTLE?.T. SEEROOT?.T.  
 MANTSURF:10 COLOUR:ye-br NEWCOLOUR:ye-br OLDLOUR:ye-br  
 COLOURNOTE:darkening with age RZFREQ:n EH?.F. ETOH:not  
 (sn82) FESO4:not KOH:not LACTICACID:not MELTZERS:not  
 SULFOVAN:not AF254:not AF366:not LACT? .T.

LACTDIST:maybe yes (yes sn 86) LACTDIAM:6  $\mu$ m LACTRAM:60 deg LACTCONT:granular or clear LACTCOLOUR:n OMANTYPE:4 OMCELLSHAP:much like figure, bit longer OMSEPTAVIS?.F. OMCELLWIDE:2  $\mu$ m OMCELLLEN:15  $\mu$ m OMCOLOUR:n OMCELLSURF:sm OMANATNOTE:thick walled 1-1.5  $\mu$ m, 3 $\mu$ m wide at ends, 6-20 long, sn86:(type 4, 2 cell layers thick) IMANTYPE:3 IMCELLSHAP:much like figure IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:3  $\mu$ m IMCELLLEN:15  $\mu$ m IMCOLOUR:n IMCELLSURF:sm IMANATNOTE:len 13-21, width 2-3, 1 obs. bit but prob typical, sn86:(type 3) EHSEPTAVIS?.F. CY?.F. SCLEROTIA?.F. MISCNOTES:distinctive

EM:58 GENUS:*Gomphidius?* SN:78 DATE:12/30/93 NTIPS:6 TTOTAL:33 CTOTAL:3 CLASS:c RAM:2 SYSLEN:8 mm TIPDIAM:530  $\mu$ m TIPSHAPE:b SEEMANTLE?.T. SEEROOT?.F. MANTSURF:15 COLOUR:ye-wh NEWCOLOUR:ye-wh OLDLOUR:ye-wh COLOURNOTE:off-white RZFREQ:a RZOCCUR:all over RZCONNECT:19 RZCOLOUR:ye-wt RZDIAM:500  $\mu$ m RZRAM:like picture of rzconnect=19 RZSURF:21 EH?.T. EHDENSITY:a EHOCCUR:all over EHLPSHAPE:rat's nest ETOH:insufficient material AF254:none AF366:none LACT?.F. OMANTYPE:1 OMSEPTAVIS?.T. OMCLAMPS?.F. OMCELLWIDE:2  $\mu$ m OMCOLOUR:n OMCELLSURF:rough--fine to a bit coarse IMANTYPE:1 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:2  $\mu$ m IMCOLOUR:n IMCELLSURF:rough RZANAT:parallel ornamented hyphae EHSEPTAVIS?.T. EHCLAMPS?.F. EHCOLOUR:n EHSURF:rough to smooth EHRAM:90 deg EHJOINTYPE:H CY?.F. SCLEROTIA?.F. MISCNOTES:like em2 but with granular detachable orn, not elongated, poor descriptions, looks like em2

EM:59 SN:78 DATE:12/30/93 NTIPS:31 TTOTAL:31 CTOTAL:2 CLASS:a RAM:5 SYSLEN:6 mm TIPDIAM:440  $\mu$ m AXDIAM:450  $\mu$ m TIPSHAPE:b MORPHNOTE:tips with several bends, sugar coated

SEEMANTLE?.T. SEEROOT?.F. MANTSURF:11 COLOUR:wh  
 NEWCOLOUR:wh OLDCOLOUR:wh COLOURNOTE:cortex visible in  
 places rubbed RZFREQ:o RZOCCUR:at base of tips  
 RZCONNECT:18 RZCOLOUR:wh RZDIAM:125 µm RZRAM:90 deg  
 RZXS:r RZSURF:22 RZNOTE:1 obs EH?.F. ETOH:sn82:n  
 FESO4:sn82:n KOH:sn82:n LACTICACID:sn82:n  
 MELTZERS:sn82:blue-green after rinsing SULFOVAN:sn82:very  
 reddish-purple after rinsing AF254:none AF366:none LACT?  
 .F. OMANTYPE:4 OMCELLSHAP:jigsaw OMSEPTAVIS?.F.  
 OMCELLWIDE:4 µm OMCOLOUR:n OMCELLSURF:sm OMANATNOTE:thin  
 mantle easily peeled, clear surface layer breaks into  
 plates, many short branches, sn84:type 4 IMANTYPE:2  
 IMSEPTAVIS?.F. IMCELLWIDE:4 µm IMCOLOUR:n IMCELLSURF:sm  
 RZANAT:surface like mantle (plates and blunt ends), most  
 4-5 inside RZVESS:few in centre, some w granular contents  
 VESSDIAM:12 EHHPSHAPE:few EHSEPTAVIS?.T. EHCLAMPS?.F.  
 EHDIAM:3 µm EHWALLTHIK:3 µm EHCOLOUR:pu EHSURF:sm  
 EHRAM:highly ramified EHPROXIMAL:blunt EHANATNOTE:purple,  
 sn82:clampless septa CY?.F. SCLEROTIA?.F.  
 MISCNOTES:surface layer breaks into plates, distinctive,  
 bit like em5, in mineral soil

EM:60 SN:79 DATE:01/03/94 NTIPS:9 TTOTAL:12 CTOTAL:2  
 CLASS:c RAM:2 SYSLIN:4 mm TIPDIAM:390 µm TIPSHAPE:b  
 SEEMANTLE?.T. SEEROOT?.F. MANTSURF:11 COLOUR:wh  
 NEWCOLOUR:wh OLDCOLOUR:wh RZFREQ:a RZOCCUR:all over  
 especially at tips RZCONNECT:19 RZCOLOUR:wh RZDIAM:50 µm  
 RZRAM:narrow angle RZXS:f RZSURF:23 RZNOTE:surf 23-21,  
 diam mostly ca. 40, to 140 EH?.T. EHDENSITY:a  
 EHOCCUR:all over EHLPSHAPE:finely hairy ETOH:insufficient  
 material AF254:none AF366:none LACT?.F. OMANTYPE:1  
 OMSEPTAVIS?.T. OMCLAMPS?.F. OMCELLWIDE:3 µm OMCOLOUR:n  
 OMCELLSURF:sm to ornamented w elongated xtls

OMANATNOTE:lots of H anastomoses, width 2.5-3 IMANTYPE:2  
 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:3  $\mu\text{m}$  IMCOLOUR:n  
 IMCELLSURF:sm to elongated xtls RZANAT:parallel hyphae,  
 some w crystals perpendicular to hyphae, 2-3  $\mu\text{m}$   
 EHHPSHAPE:much like outer mantle EHSEPTAVIS?.T.  
 EHCLAMPS?.F. EHDIAM:3  $\mu\text{m}$  EHCLOUR:n EHSURF:mostly  
 smooth, some with granular to elongated xtl EHRAM:90 deg  
 CY?.F. SCLEROTIA?.F. MISCNOTES:looks like em55 but with  
 some elongated xtls and smaller hyphae, poor description,  
 indistinct

EM:61 SN:81 DATE:01/04/94 NTIPS:42 TTOTAL:42 CTOTAL:1  
 CLASS: RAM:5 SYLEN:9 mm TIPDIAM:410  $\mu\text{m}$  AXDIAM:470  $\mu\text{m}$   
 TIPSHAPE:b MORPHNOTE:long tips with many bends  
 SEEMANTLE?.T. SEEROOT?.F. MANTSURF:11 COLOUR:wh  
 NEWCOLOUR:wh OLDLOUR:br-ye COLOURNOTE:apices grey,  
 swollen, bits of lt-br-ye show thru RZFREQ:a RZOCUR:all  
 over RZCONNECT:19 RZCOLOUR:wh RZDIAM:110  $\mu\text{m}$  RZRAM:30-60  
 deg RZXS:r RZSURF:22 RZNOTE:not tough, but not loose  
 EH?.T. EHDENSITY:c EHOCUR:all over EHLPSHAPE:cob-webby  
 ETOH:not FES04:not KOH:not LACTICACID:not MELTZERS:not  
 SULFOVAN:not AF254:not AF366:not LACT? .F. OMANTYPE:1  
 OMSEPTAVIS?.T. OMCLAMPS?.T. OMSEPTANOT:some septa not  
 clamped OMCELLWIDE:4  $\mu\text{m}$  OMCOLOUR:n OMCELLSURF:sm  
 OMANATNOTE:widths3-7, outer hyphae largest, very  
 thin-walled IMANTYPE:3 IMSEPTAVIS?.T. IMCLAMPS?.T.  
 IMSEPTANOT:some septa not clamped IMCELLWIDE:4  $\mu\text{m}$   
 IMCOLOUR:n IMCELLSURF:sm IMANATNOTE:some areas of  
 parallel hyphae, widths 3-5 mostly EHSEPTAVIS?.T.  
 EHCLAMPS?.T. EHSEPTANOT:some septa not clamped EHDIAM:4  
 $\mu\text{m}$  EHCLOUR:n EHSURF:sm CY?.F. SCLEROTIA?.F.  
 MISCNOTES:fairly loose, very thin mantle 2 layers? like  
 em10 but not fluorescent, one observation only

EM:62 GENUS:*Russula* SPECIES:*aeruginea* SN:88 DATE:03/07/94  
 NTIPS:5 TTOTAL:230 CTOTAL:6 CLASS:a RAM:5 SYSLEN:12 mm  
 TIPDIAM:484  $\mu$ m TIPSHAPE:c SEEMANTLE?.T. SEEROOT?.T.  
 MANTSURF:12 COLOUR:br NEWCOLOUR:wt OLDCOLOUR:br  
 COLOURNOTE:old tips brown or white (air trapped in  
 cystidia) RZFREQ:n EH?.F. ETOH:insufficient material  
 AF254:n AF366:n LACT? .F. OMATYPE:2 OMSEPTAVIS?.T.  
 OMCLAMPS?.F. OMCELLWIDE:4  $\mu$ m OMCELLSURF:sm  
 OMANATNOTE:thin or thick(sn103), network from clumps, hard  
 to see IMATYPE:3 IMSEPTAVIS?.T. IMCLAMPS?.F.  
 IMCELLWIDE:3  $\mu$ m IMCOLOUR:n IMCELLSURF:sm  
 IMANATNOTE:groups of parallel hyphae at right angles  
 EHPHSHAPE:sn103 wiggly a bit EHSEPTAVIS?.T. EHCLAMPS?.F.  
 EHDIAM:2  $\mu$ m EHANATNOTE:none in sn88 CY?.T.  
 CYSHAPE:bowling pin/club, droplet at tip CYLEN:25  $\mu$ m  
 CYWIDTH:4  $\mu$ m CYCONT:granular or not CYCOLOUR:none  
 CYSURF:sm CYANATNOTE:occasionally septate, droplet or  
 spore at tip, sn103:(commonly 40 long, some with inflated  
 bases (6-10)) SCLEROTIA?.F. MISCNOTES:distinctive

EM:63 SN:82 DATE:01/25/94 NTIPS:29 TTOTAL:67 CTOTAL:2  
 CLASS:b RAM:5 SYSLEN:5 mm TIPDIAM:425  $\mu$ m TIPSHAPE:b  
 SEEMANTLE?.T. SEEROOT?.F. MANTSURF:11 COLOUR:lt-or  
 NEWCOLOUR:lt-or OLDCOLOUR:lt-or COLOURNOTE:variable,  
 parts white, bits have black cortex showing thru, check  
 sn102 RZFREQ:a RZOCCUR:all over RZCONNECT:20  
 RZCOLOUR:or RZDIAM:250  $\mu$ m RZXS:f RZSURF:21  
 RZNOTE:orange or white 200-300  $\mu$ m EH?.T. EHDENSITY:c  
 EHOCCUR:all over EHMORPNOTE:variable density  
 ETOH:insufficient material AF254:n AF366:n LACT? .F.  
 OMATYPE:2 OMSEPTAVIS?.T. OMCLAMPS?.F. OMCELLWIDE:3  $\mu$ m  
 OMCOLOUR:or OMCELLSURF:finely ornamented OMANATNOTE:some  
 orange contents, hyphae 2-3 $\mu$ m, a few larger hyphae 4-5 $\mu$ m,

sn102: thin with black cortex IMANTYPE:3  
 IMCELLSHAP:groups of parallel hyphae IMSEPTAVIS?.T.  
 IMCLAMPS?.F. IMCELLWIDE:3 µm IMCOLOUR:or IMCELLSURF:sm  
 IMANATNOTE:some orange? mostly 2-2.5, some 3  
 EHHPSHAPE:straight but with slightly uneven walls  
 EHSEPTAVIS?.T. EHCLAMPS?.F. EHDIAM:2 µm EHCOLOUR:or  
 EHSURF:smooth, some finely ornamented EHANATNOTE:mostly  
 colourless CY?.F. SCLEROTIA?.F. MISCNOTES:orange!, two  
 observations, variable descriptions, put with em68

EM:64 SN:82 DATE:01/25/94 NTIPS:17 TTOTAL:17 CTOTAL:1  
 CLASS:b RAM:0 SYLEN:9 mm TIPDIAM:510 µm AXDIAM:620 µm  
 TIPSHAPE:b MORPHNOTE:some tips with many bends, couldn't  
 tell ramification, surface also fuzzy SEEMANTLE?.T.  
 SEEROOT?.T. MANTSURF:12 COLOUR:wh NEWCOLOUR:lt-ye  
 OLDCOLOUR:wh COLOURNOTE:apices lemon, brown patches common  
 RZFREQ:n EH?.T. EHDENSITY:a EHOCCUR:all over  
 EHLPSHAPE:fuzzy ETOH:n FESO4:n KOH:n LACTICACID:n  
 MELTZERS:n SULFOVAN:n AF254:n AF366:n LACT? .F.  
 OMANTYPE:1 OMSEPTAVIS?.T. OMCLAMPS?.F. OMCELLWIDE:3 µm  
 OMCOLOUR:n OMCELLSURF:sm OMANATNOTE:very thick, hard to  
 see for eh IMANTYPE:3 IMSEPTAVIS?.T. IMCLAMPS?.F.  
 IMCELLWIDE:3 µm IMCOLOUR:n IMCELLSURF:sm  
 EHHPSHAPE:wiggly, moderately strait with wiggly walls  
 EHSEPTAVIS?.T. EHCLAMPS?.F. EHDIAM:3 µm EHCOLOUR:n  
 EHSURF:sm EHRAM:60 degrees CY?.F. SCLEROTIA?.F.  
 MISCNOTES:not 52 because of wider hyphae, few rz, lots eh,  
 non-descript, one observation

EM:65 SN:92 DATE:03/14/94 NTIPS:53 TTOTAL:150 CTOTAL:4  
 CLASS:a RAM:2 SYLEN:13 mm TIPDIAM:530 µm AXDIAM:680 µm  
 TIPSHAPE:b MORPHNOTE:tipshapeb-c, finely woolly, or felty,  
 38 tips sn104 SEEMANTLE?.T. SEEROOT?.F. MANTSURF:11

COLOUR:lt-br NEWCOLOUR:lt-br OLDCOLOUR:br  
 COLOURNOTE:sn104: lt-br, wh, ye-br, or very light brown  
 almost white, with yellowish tints RZFREQ:n EH?.F.  
 ETOH:n FESO4:n KOH:whitish after rinsing AF254:n  
 AF366:n LACT?.F. OMANTYPE:2 OMSEPTAVIS?.T.  
 OMCLAMPS?.T. OMCELLWIDE:4 µm OMCOLOUR:n OMCELLSURF:sm  
 OMANATNOTE:granular contents, 3-4.5 wide hyphae, thick  
 mantle sn104:(oily contents, type 3-5, thick mantle)  
 IMANTYPE:3 IMCELLSHAP:some round to polygonal  
 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:5 µm IMCOLOUR:n  
 IMCELLSURF:sm IMANATNOTE:width 3-8, sn104 3-6  
 EHHPSHAPE:like outer mantle EHSEPTAVIS?.F.  
 EHANATNOTE:like outer mantle CY?.F. SCLEROTIA?.F.

EM:66 SN:107 DATE:06/13/94 NTIPS:26 TTOTAL:26 CTOTAL:1  
 CLASS:c RAM:2 SYLEN:6 mm TIPDIAM:500 µm AXDIAM:570 µm  
 TIPSHAPE:a MORPHNOTE:mantle brown in places, but generally  
 the cortex is not visible, almost stringy SEEMANTLE?.T.  
 SEEROOT?.F. MANTSURF:11 COLOUR:br+wh NEWCOLOUR:br+wh  
 OLDCOLOUR:br+wh COLOURNOTE:apices lighter, white on brown,  
 mostly br but more like wh types than br type RZFREQ:c  
 RZOCCUR:no usually at tip RZCONNECT:19 RZCOLOUR:wh  
 RZDIAM:150 µm RZRAM:gradual RZXS:r RZSURF:23  
 RZNOTE:parallel hyphae, diam 30-280 EH?.T. EHDENSITY:a  
 EHOCCUR:all over EHLPSHAPE:in fans AF254:n AF366:n  
 LACT?.F. OMANTYPE:2 OMCELLSHAP:straightish  
 OMSEPTAVIS?.T. OMCLAMPS?.T. OMSEPTANOT:not quite  
 hemispherical clamps OMCELLWIDE:3 µm OMCOLOUR:n  
 OMCELLSURF:sm OMANATNOTE:some granular contents, 2.5-3.5  
 IMANTYPE:3 IMCELLSHAP:like fig2.3 but longer  
 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:4 µm IMCELLLEN:24  
 µm IMCOLOUR:n IMCELLSURF:s IMANATNOTE:in parallel  
 bundles of 15-30, 1.5-7X6-50 RZANAT:parallel clamped

hyphae, ca. 4µm EHHPSHAPE:straight EHSEPTAVIS?.T.  
 EHCLAMPS?.T. EHSEPTANOT:some swollen EHDIAM:3 µm  
 EHCLOUR:n EHSURF:s EHANATNOTE:2-4 µm CY?.F.  
 SCLEROTIA?.F. MISCNOTES:v. much like em 16, but stringier,  
 browner, hard to examine, white type, one observations

EM:67 SN:108 DATE:06/14/94 NTIPS:5 TTOTAL:5 CTOTAL:1  
 CLASS:c RAM:2 SYLEN:10 mm TIPDIAM:720 µm TIPSHAPE:a  
 MORPHNOTE:woolly mantle, mantle surface not very distinct  
 SEEMANTLE?.T. SEEROOT?.F. MANTSURF:13 COLOUR:wh  
 NEWCOLOUR:wh OLDLOUR:wh RZFREQ:a RZOCCUR:all over  
 RZCONNECT:21 RZCOLOUR:wh RZDIAM:58 µm RZRAM:gradual  
 RZXS:f RZSURF:21 RZNOTE:diam 30, 85 EH?.F.  
 ETOH:insufficient material AF254:n AF366:n LACT? .F.  
 OMATYPE:2 OMSEPTAVIS?.T. OMCLAMPS?.T. OMCELLWIDE:4 µm  
 OMCOLOUR:n OMCELLSURF:finely and sparsely ornamented  
 OMANATNOTE:not densely orn like em27, width 3-4, not all  
 septa clamped IMATYPE:3 IMSEPTAVIS?.T. IMCLAMPS?.F.  
 IMCELLWIDE:4 µm IMCOLOUR:n IMCELLSURF:s  
 EHHPSHAPE:straight EHSEPTAVIS?.T. EHCLAMPS?.T. EHDIAM:3  
 µm EHCLOUR:n EHSURF:orn as for om CY?.F. SCLEROTIA?.F.  
 MISCNOTES:clear features, but rare

EM:68 GENUS:Hydnellum? SPECIES:peckii? (Agerer 1993)  
 SN:109 DATE:06/21/94 NTIPS:14 TTOTAL:14 CTOTAL:1  
 CLASS:b RAM:5 SYLEN:7 mm TIPDIAM:580 µm AXDIAM:690 µm  
 TIPSHAPE:b MORPHNOTE:felted mantle SEEMANTLE?.T.  
 SEEROOT?.F. MANTSURF:11 COLOUR:wh NEWCOLOUR:wh  
 OLDLOUR:lt-ye COLOURNOTE:older parts maybe black,  
 carbonizing mantle RZFREQ:a RZOCCUR:all over  
 RZCONNECT:19 RZCOLOUR:wh RZDIAM:200 µm RZXS:f RZSURF:21  
 RZNOTE:sheet-like with lots of eh between, very flat in  
 cross-section EH?.T. EHDENSITY:a EHOCCUR:all over

EHMORPNOTE:along with rz ETOH:n FESO4:n KOH:n  
 LACTICACID:n MELTZERS:n SULFOVAN:n AF254:n AF366:n  
 LACT? .F. OMANTYPE:2 OMSEPTAVIS?.T. OMCLAMPS?.F.  
 OMCELLWIDE:2 µm OMCOLOUR:n OMCELLSURF:finely ornamented  
 IMANTYPE:3 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:3 µm  
 IMCOLOUR:n IMCELLSURF:s IMANATNOTE:2-3 µm wide  
 EHSEPTAVIS?.F. EHANATNOTE:much like outer mantle CY?.F.  
 SCLEROTIA?.F. MISCNOTES:chlamydo spores about 5 µm, one  
 observation only

EM:69 GENUS:*Lactarius* SN:111 DATE:06/16/94 NTIPS:33  
 TTOTAL:33 CTOTAL:1 CLASS:a RAM:2 SYLEN:15 mm  
 TIPDIAM:465 µm AXDIAM:610 µm TIPSHAPE:b MORPHNOTE:finely  
 grainy, mat SEEMANTLE?.T. SEEROOT?.T. MANTSURF:12  
 COLOUR:br NEWCOLOUR:lt-ye OLDLOUR:br RZFREQ:n EH?.F.  
 ETOH:n FESO4:n KOH:after rinsing orange air patches  
 LACTICACID:n MELTZERS:n SULFOVAN:n AF254:n AF366:n  
 LACT? .T. LACTDIST:all over LACTDIAM:4 µm  
 LACTCONT:granular to clear LACTCOLOUR:n OMANTYPE:4  
 OMCELLSHAP:jigsaw, potato OMSEPTAVIS?.F. OMCELLWIDE:7 µm  
 OMCELLLEN:15 µm OMCOLOUR:n OMCELLSURF:s  
 OMANATNOTE:16x10,10x15,21x14,20x6,5x5 IMANTYPE:3  
 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:3 µm  
 IMANATNOTE:2.5-3 µm wide EHSEPTAVIS?.F. CY?.F.  
 SCLEROTIA?.F. MISCNOTES:distinctive due to laticiferous  
 hyphae

EM:70 SN:114 DATE:06/24/94 NTIPS:155 TTOTAL:155  
 CTOTAL:1 CLASS:b RAM:2 SYLEN:7 mm TIPDIAM:510 µm  
 AXDIAM:485 µm TIPSHAPE:b MORPHNOTE:all tips senescent  
 carbonizing SEEMANTLE?.T. SEEROOT?.F. MANTSURF:11  
 COLOUR:wh NEWCOLOUR:wh OLDLOUR:br COLOURNOTE:white  
 fades and turns brown RZFREQ:a RZOCCUR:all over

RZCONNECT:0 RZCOLOUR:ye-wh RZDIAM:400 µm RZRAM:30  
 degrees, frequent RZXS:r RZSURF:23 RZNOTE:rz dont seem  
 to be attached, same fungus??, fleshy EH?.F. ETOH:n  
 FESO4:n KOH:n LACTICACID:n MELTZERS:n SULFOVAN:n  
 AF254:n AF366:n LACT? .F. OMANTYPE:2  
 OMCELLSHAP:tapering OMSEPTAVIS?.T. OMCLAMPS?.F.  
 OMCELLWIDE:2 µm OMCOLOUR:n OMCELLSURF:s OMANATNOTE:in  
 bunches radiating IMANTYPE:3 IMSEPTAVIS?.T. IMCLAMPS?.F.  
 IMCELLWIDE:2 µm IMCOLOUR:n IMCELLSURF:s  
 IMANATNOTE:parallel hyphae RZANAT:parallel hyphae, no  
 clamps, same fungus? no connex EHSEPTAVIS?.F. CY?.F.  
 SCLEROTIA?.F. MISCNOTES:could be em64, one observation

EM:71 SN:120 DATE:06/29/94 NTIPS:10 TTOTAL:42 CTOTAL:1  
 CLASS:b RAM:2 SYLEN:15 mm TIPDIAM:460 µm AXDIAM:550 µm  
 TIPSHAPE:b MORPHNOTE:brown patches where cortex seen  
 SEEMANTLE?.T. SEEROOT?.F. MANTSURF:11 COLOUR:br-wh  
 NEWCOLOUR:br-wt OLDCOLOUR:br-wt COLOURNOTE:off white,  
 pinkish tinge RZFREQ:a RZOCUR:all over RZCONNECT:18  
 RZCOLOUR:br-wt RZDIAM:35 µm RZRAM:30,90, etc degrees  
 RZXS:r RZSURF:22 RZNOTE:brown patches on rz EH?.T.  
 EHDENSITY:a EHOCCUR:all over EHLPSHAPE:wispy ETOH:n  
 FESO4:n KOH:n LACTICACID:n MELTZERS:n SULFOVAN:n  
 AF254:n AF366:n LACT? .F. OMANTYPE:1 OMSEPTAVIS?.T.  
 OMCLAMPS?.F. OMCELLWIDE:1 µm OMCOLOUR:gr OMCELLSURF:sm  
 OMANATNOTE:very thin, 1 layer plus surface hyphae  
 IMANTYPE:3 IMSEPTAVIS?.T. IMCLAMPS?.F. IMCELLWIDE:2 µm  
 IMANATNOTE:width 2,2,2,3,4,2,2,2 RZANAT:woven, narrow  
 thick walled hyphae EHSEPTAVIS?.F. EHDIAM:1 µm  
 EHWALLTHIK:1 µm EHANATNOTE:like om CY?.F. SCLEROTIA?.F.  
 MISCNOTES:looks like rhizopogon, like em46 but for hyphae.  
 parasitic?, or early stage of development of something?

### Appendix 1B. Key to ectomycorrhizas

1. a) fluorescent under UV light (254 or 366 nm):  
FLUORESCENT.
- b) not fluorescent: 2.
2. a) with laticiferous hyphae: *Lactarius*.
- b) without laticiferous hyphae: 3.
3. a) tuberculate: TUBERCULATE.
- b) not tuberculate: 4.
4. a) distinctively yellow: YELLOW.
- b) light yellow or not yellow: 5.
5. a) dark, black or brown: DARK.
- b) light coloured, white, pink, purple, blue, orange,  
brown or light brown: 6.
6. a) most areas of mantle surface white or nearly white  
except where bruised or damaged, surface of cortex may  
be partially visible if the mantle is thin : WHITE.
- b) mainly without white surface colour: BROWN, ORANGE,  
RED, OR PALE YELLOW.

#### FLUORESCENT

- a) white, turns dark in reaction to Meltzer's reagent,  
yellow under UV: **em10**.
- b) light pink, rhizomorphs or mantle turn blue in KOH,  
yellow under UV: **em46**.

#### *Lactarius*

1. a) brown with green patches, latex orange, outer  
mantle a net synenchyma: **em13**, *Lactarius deliciosus*  
(Fr.) S.F.G..
- b) without orange or green pigment, outer mantle an  
interlocking irregular synenchyma: 2.
2. a) cells of mantle surface 2-3  $\mu\text{m}$  wide, thick-walled:

**em57, *Lactarius*.**

b) cells of mantle surface 4-10  $\mu\text{m}$  wide, not thick-walled: **em69, *Lactarius*.**

## TUBERCULATE

abundant rhizomorphs attached to brown-silver peridium, dark brown narrow aseptate felty mycelium under peridium: **em7**, like *Rhizopogon vinicolor* A.H. Smith.

## YELLOW

a) bright yellow, clampless, finely ornamented hyphae in mantle: **em3, *Piloderma fallax*** (Libert) Stalpers.

b) brownish yellow, clamps, abundant rhizomorphs with emanating hyphae: **em20**, like *Amphinema byssoides* (Pers.: Fr.) Erikss. (Agerer 1993, Ingleby et al. 1990)

c) bright yellow, slightly greenish, occasional smooth-surfaced rhizomorphs, clamped emanating hyphae: **em29**.

## DARK

1. a) shiny black with long dark setae: **em1, *Cenococcum geophilum*** Fr..

b) dark brown to black, with rough surface: 2.

2. a) with setae: 3.

b) without setae: 7.

3. a) with short setae, <50  $\mu\text{m}$ : 4.

b) with long setae (80  $\mu\text{m}$ ): 6.

4. a) all setae elongated: 5.

b) some setae triangular and roughly equilateral, others short and sharp-tipped, mycorrhizae also may be light brown: **em14**.

5. a) curved setae with bulbous bases: **em28**, like "*Fagirhiza spinulosa*" (Agerer 1993).

- b) triangular setae without bulbous bases: **em45**.
- 6. a) outer mantle of regular synenchyma: **em44**, like *Tuber*.  
b) outer mantle prosenchymatous: **em34**.
- 7. a) finely grainy, hyphae in outer mantle about 5  $\mu\text{m}$  diam.: **em9**.  
b) hyphae in outer mantle about 2  $\mu\text{m}$  diam.: 8.
- 8. a) dark brown, outer mantle a clearly visible prosenchymatous network of hyphae with short hornlike side branches, hyphae of inner mantle very thick walled, highly convoluted in a brain-like pattern: **em41**, like "*Piceirhiza bicolorata*" (Agerer 1993).  
b) dark brown with a greenish tinge, outer mantle with few visible hyphae, hyphae unlike those of em 41: **em38**.

## WHITE

- 1. a) white to light orange, hyphae finely ornamented: **em63**.  
b) no orange colour: 2.
- 2. a) surface of mantle and rhizomorphs covered with a thin transparent layer that appears to break into plates: **em59**.  
b) emanating hyphae common, surface of mantle and rhizomorphs without transparent plates: 3.
- 3. a) clamp connections abundant on hyphae in outer layer of mantle or on emanating hyphae: 4.  
b) clamp connections absent or rare on septa of hyphae in outer mantle: 11.
- 4. a) rhizomorphs fleshy, or at least somewhat cohesive, round in cross-section: 5.  
b) rhizomorphs loosely-knit, maybe flat in cross-section: 6.
- 5. a) rhizomorphs dense, fleshy, with restricted point of attachment to mantle, hyphae of outer mantle 3  $\mu\text{m}$  diam:

**em37.**

- b) rhizomorphs not as dense, growing off mantle at narrow angle with extended area of attachment, hyphae of outer mantle 3-7  $\mu\text{m}$ : **em61.**
6. a) at least a few hyphae ornamented or with surface deposits (emanating hyphae or hyphae in the mantle or in rhizomorphs): 7.  
 b) all hyphae of outer mantle, rhizomorphs, and emanating hyphae smooth: 8.
7. a) ornamentation uniform in size and distribution within 10 $\mu\text{m}$  lengths of hyphae: **em27.**  
 b) ornamentation varied in size and distribution within 10 $\mu\text{m}$  length of hyphae: **em67**, like *Amanita muscaria* (Fr.) S.F. Gray var. *formosa* (link).
8. a) rhizomorphs very loose, intergrading with hyphae, up to 500 $\mu\text{m}$  wide, hyphae in outer mantle without granular contents, mostly 4-5  $\mu\text{m}$  diameter: **em8.**  
 b) rhizomorphs < 200  $\mu\text{m}$  wide, distinct from emanating hyphae, hyphae in outer mantle mostly 3-4  $\mu\text{m}$  wide: 9.
9. a) with swollen clamp connections: 10.  
 b) hyphae without swollen clamp connections: **em11.**
10. a) mantle reticulate, not stringy, hyphae of outer mantle 3-5  $\mu\text{m}$  wide: **em16.**  
 b) mantle reticulate and stringy in places, hyphae 2-3  $\mu\text{m}$  wide in outer mantle: **em66.**
11. a) hyphae of outer mantle and emanating hyphae smooth: 19.  
 b) hyphae of outer mantle or emanating hyphae with crystalline or globular deposits, or ornamentation: 12.
12. a) with club-shaped cystidia: **em54.**  
 b) without cystidia: 13.
13. a) with finely ornamented hyphae in outer mantle, emanating from mantle and in rhizomorphs, some hyphae coarsely ornamented: 14.

- b) hyphae with elongated crystalline or globular surface deposits: 17.
14. a) rhizomorphs mostly 50  $\mu\text{m}$ , connected to mantle at restricted points, emanating hyphae not conspicuous: **em49**.  
b) rhizomorphs mostly 100-500  $\mu\text{m}$ , connected to mantle at wide angles, conspicuous emanating hyphae: 15.
15. a) hyphae of outer mantle 4-5  $\mu\text{m}$  wide, mantle thin, much of host is visible: **em55**.  
b) hyphae of outer mantle and rhizomorphs 2  $\mu\text{m}$  wide, mantle thick, host surface not visible: 16.
16. a) carbonizing, with chlamydospores: **em68**, like *Hydnellum peckii* Banker apud Peck (Agerer 1993).  
b) not carbonizing, without chlamydospores: **em58**, like *Gomphidius* (link).
17. a) rhizomorphs commonly 150  $\mu\text{m}$ , hyphae of outer mantle 2-3  $\mu\text{m}$ , some hyphae with elongated crystals, no granular bodies in outer mantle, no colour reaction to bruising, no clamps: 18.  
b) rhizomorphs 50  $\mu\text{m}$ , mantle stains or bruises brownish yellow, hyphae of outer mantle 2-5  $\mu\text{m}$  in diameter, some hyphae of outer mantle and emanating hyphae with large or small spherical surface deposits or exudates, may have abundant granular bodies on mantle surface, some clamp connections in rhizomorphs or emanating hyphae: **em39**.
18. a) slightly yellowish with age, with elongated crystals on surface of hyphae: **em2**, like *Hysterangium*.  
b) with both granular and elongated crystalline deposits on surface of hyphae: **em60**, like *Gomphidius* (link).
19. a) outer layer of mantle synenchymatous in places, or else a net prosenchyma, inner layer a net prosenchyma or a net synenchyma: **em19**.  
b) outer layer of mantle prosenchymatous, inner layer

- a net synenchyma: 20.
20. a) rhizomorphs common, apices white or brownish white: 21.  
b) rhizomorphs absent to uncommon, apices white or pale yellow: **em64**.
21. a) outer surface of mantle with granular inclusions obscuring hyphae: 24.  
b) mantle surface without numerous inclusions: 22.
22. a) mantle very thin, hyphae of outer mantle 1  $\mu\text{m}$  wide: **em71**.  
b) mantle not very thin, hyphae of mantle surface hyphae of mantle surface 1-3  $\mu\text{m}$  wide: 23.
23. a) mantle turning brownish with age, emanating hyphae absent: **em70**.  
b) mantle remaining white or becoming transparent with age, emanating hyphae common: **em26**.
24. a) hyphae on surface of rhizomorphs convoluted and branched, similarly to *Byssoporia* (Zak and Larsen 1978), mantle bruising blue, turning purple in sulfovanillin, cells of inner mantle 2  $\mu\text{m}$  wide: **em5**.  
b) hyphae of rhizomorph surface not convoluted, mantle without blue colour, no colour reaction to sulfovanillin, cells of inner mantle 3-5(6)  $\mu\text{m}$  wide: **em52**.

BROWN, ORANGE, RED OR PALE YELLOW

1. a) outer layer of mantle prosenchymatous throughout: 2.  
b) outer layer of mantle synenchymatous, at least in places: 13.
2. a) mycorrhizas smooth or very nearly so when viewed at 40X, with the possible exception of older parts, septa clampless, few to no rhizomorphs: 3.  
b) surface of mycorrhizas appear distinctly textured when viewed at 40X magnification: 6.

3. a) thick transparent mantle of narrow (1-2  $\mu\text{m}$ ) hyphae apparently gelatinous matrix, inner mantle prosenchymatous: **em24**.  
b) mantle not uncommonly thick, hyphae of outer layer of mantle mostly 3  $\mu\text{m}$  or larger: 4.
4. a) hyphae of outer layer of mantle about 5  $\mu\text{m}$  wide, some with bulbous tips 10-15  $\mu\text{m}$ : **em4**.  
b) hyphae of outer mantle 2-4  $\mu\text{m}$  wide, without bulbous tips: 5.
5. a) light brown, mantle shiny cells of inner mantle 1.5  $\mu\text{m}$  wide: **em51**.  
b) light brown with orange tints, matte rather than shiny, mantle brittle as evidenced by cracks, soil particles frequently attached to mantle, cells of inner mantle 2-3  $\mu\text{m}$  wide, cells of outer mantle very hyaline and not easily seen: **em6**.
6. a) surface of cortex not visible, due to surface pigmentation or colour: 7.  
b) surface of cortex visible to large extent thru the mantle: 10.
7. a) mass of emanating hyphae obscures tips: **em18**, like *Gomphidius glutinosus* (Schaeff.: Fr.) Fr. (link).  
b) individual tips clearly visible: 8.
8. a) surface felty, hyphae in outer mantle smooth with some clamps: 9.  
b) surface grainy, not felty, septa not clamped, hyphae in outer mantle ornamented, brown colour in mantle due to hyphal exudates: **em30**.
9. a) coarsely felty, hyphae of outer mantle 2-3  $\mu\text{m}$  wide and without granular contents: **em15**.  
b) finely felty, hyphae of outer mantle 3-5  $\mu\text{m}$  wide with granular contents: **em65**.
10. a) clamps on hyphae of outer mantle and emanating hyphae: **em22**.  
b) hyphae of outer mantle and emanating hyphae without

- clamps: 11.
11. a) with thin-walled cystidia with spore-like bulges at tips: **em62**, like *Russula aeruginea* Lindblad: Fr. (Agerer 1993).  
b) without cystidia: 12.
  12. a) abundant narrow (1 $\mu$ m) emanating hyphae forming cottony mass, hyphae of outer mantle 3  $\mu$ m wide: **em48**.  
b) emanating hyphae not forming a cottony mass, rather joining in loosely organized rhizomorphs, hyphae of outer mantle 1.5-2.5  $\mu$ m: **em23**.
  13. a) with cystidia on mantle surface: 14.  
b) without cystidia or setae: 18.
  14. a) cystidia > 50  $\mu$ m long: 15.  
b) setae < 30  $\mu$ m long: 17.
  15. a) cystidia septate: 16.  
b) cystidia aseptate: **em40**.
  16. a) cystidia curved, mycorrhiza surface fuzzy due to abundance of cystidia: **em31**.  
b) cystidia straight or nearly so, mycorrhiza appears smooth when viewed at 40X: **em17**.
  17. a) setae pigmented, emanating hyphae 4  $\mu$ m: **em14**.  
b) setae not pigmented, emanating hyphae 8  $\mu$ m: **em42**.
  18. a) outermost layer of mantle a non-interlocking irregular synenchyma, or a regular synenchyma: 22.  
b) outermost layer of mantle an interlocking irregular synenchyma or a net synenchyma: 19.
  19. a) mantle surface smooth, mantle very thin: 20.  
b) mantle surface textured (viewed at 40X), not extremely thin: 21.
  20. a) emanating hyphae copious, forming woolly masses, clamped, 4-5  $\mu$ m diameter, outer mantle of interlocking irregular synenchyma: **em43**.  
b) emanating hyphae absent or at least not abundant, outer mantle a net synenchyma: **em35**.
  21. a) outer mantle an irregular synenchyma, sometimes

- interlocking, sometimes not, mantle surface finely grainy: **em25**.
- b) outer mantle layer a net synenchyma, mantle surface finely felty or fuzzy: **em33**.
22. a) with hyphal scales or warts: 23.  
b) without warts, smooth: 24.
23. a) brown with white areas due to surface hyphae on older parts and at points of ramification, tips straight to bent, warts abundant: **em12**.  
b) brown with thin white surface layer in places reminiscent of powdery mildew, and thicker white surface texture towards tips, tips straight to bent: **em32**.  
c) brown without white areas, tips tortuous: **em50**.
24. a) not distinctly orange: 25.  
b) orangish-brown or brownish-orange, no emanating hyphae, cells of outer mantle predominantly roundish (non-interlocking irregular synenchyma), tips bent but not tortuous, surface shiny: **em36**.
25. a) light (pale) orange, sheets of emanating hyphae in soil, outer layer of mantle predominantly a regular synenchyma, tips tortuous, mantle surface matt: **em21**.  
b) without sheets of em, narrow clampless emanating hyphae: **em56**.

**Appendix 1C. Descriptions of ectomycorrhizas that are either distinctive, or are common with consistent features**

The following descriptions include the more important distinguishing features, and notes on how certain types can be recognized under the dissection microscope.

**em1 (*Cenococcum geophilum* Fr.):** shiny, black, with long, thick, dark brown setae; variable in size and density of setae, but always black; often with extensive dark mycelium or black spherical sclerotia in soil; outer mantle a net synenchyma with cells arranged in a stellate pattern with centrally attached setae; dead tips with a dull surface or shrivelled appearance; the inside of live tips is often soft, white and juicy; easily recognized by its shiny black surface; Figures 1.7a-c.

**em2 (*Hysterangium* Vitt.):** white to slightly yellowish with age, mantle of felt prosenchyma, rhizomorphs abundant, wide and broadly attached to mantle; hyphae narrow (2  $\mu\text{m}$ ), clampless and usually with abundant deposits of elongated crystals, especially on older tips; hyphae frequently joined by H-shaped anastomoses; can with practice be recognized by the cheesecloth-like surface texture and slightly yellowish tint of older tips, broad rhizomorphs and thick mantle; recognition confirmed by the hyphal characters; Figures 1.7d-h.

**em3 (*Piloderma fallax* (Libert) Stalpers):** bright, slightly brownish yellow, mantle of felt prosenchyma, rhizomorphs thick, abundant and broadly attached to mantle, hyphae 3  $\mu\text{m}$ , clampless with fine rounded ornamentation; occasionally, parts of the mantle or entire tips are colourless; easily recognized by colour and rhizomorphs; Figures 1.4a, 1.8a-c.

**em5:** white, sometimes with blue bruising or localized blue colour, surface texture grainy, outer mantle a net prosenchyma with many short branches and convoluted hyphae; rhizomorphs common, round, branching at angles 60 degrees or wider, with some blue stains, surface hyphae narrow (1-2  $\mu\text{m}$ ) and highly convoluted; purple reaction to sulfovanillin; may not always show the blue and purple colour reactions, defined by mantle and rhizomorph characters; Figures 1.4b, 1.8d, 1.8e.

**em6:** light brown, plump, frequently branched, monopodially pinnate branching, smooth and matte to slightly felty with age, outer mantle a net prosenchyma, hyphae of mantle surface are difficult to see in an apparently gelatinous matrix; mantle brittle, frequently cracked, with organic soil particles commonly firmly embedded in the surface of the mantle; tentatively recognizable at 40X by colour, texture, cracking, branching and tendency to hold soil particles; Figures 1.4c, 1.8f, 1.8g.

**em7 (*Rhizopogon vinicolor* A.H. Smith):** tips aggregated in roughly isodiametric tubercles 2-20 mm in diameter, tubercles connected to one root and up to 10 rhizomorphs, even very small systems of two or more tips are already wrapped in a thin peridium of characteristic 2  $\mu\text{m}$  wide dark, slightly reddish or yellowish brown hyphae; with age the outer surface of the peridium becomes silvery grey; rhizomorphs round, with larger, hyaline, vessel-like hyphae inside a surface layer very similar to the peridium of tubercles; rhizomorphs frequently branched, abundant in fragmented layer of soil; tips white, mantle a net prosenchyma or synenchyma; easily recognized by its tuberculate form and dark brown hyphae lining the inside of the peridium; Figures 1.4d, 1.8h, 1.9a, 1.9b.

**em8:** entirely white with abundant emanating hyphae that intergrade with rhizomorphs up to 500  $\mu\text{m}$  wide, reticulate surface, outer mantle a felt prosenchyma, hyphae 4-5  $\mu\text{m}$ , hyaline, smooth, frequently septate, clamped, cells of inner mantle 5-9  $\mu\text{m}$  wide and 9-22  $\mu\text{m}$  long; tentatively recognizable at 40X by its very whiteness, abundant emanating hyphae, and rhizomorphs, id confirmed by large, clamped, smooth hyphae; Figures 1.9c, 1.9d.

**em9:** dark brown, rough surface, outer mantle a net prosenchyma of 5  $\mu\text{m}$  clamped, wiggly and finely ornamented to smooth hyphae; distinguished from other dark brown types without cystidia by its large clamped hyphae and lack of greenish colouration; Figures 1.9f-1.9h, 1.10a.

**em10:** white to slightly yellowish, fluorescing bright yellow under 366 nm UV light and paler yellow under 254 nm; mantle and rhizomorphs turn dark in Meltzer's reagent; abundant, broadly attached rhizomorphs, prosenchymatous with smooth, clamped, 4  $\mu\text{m}$  hyphae; tentatively recognizable by texture and shape of rhizomorphs, confirmed by fluorescence; Figure 1.10b.

**em12:** light brown with white surface texture due to flakes or 'warts', old parts may be grey; may have some narrow, flat, short rhizomorphs that quickly divide into emanating hyphae; outer mantle a net prosenchyma of 5  $\mu\text{m}$  hyphae or a synenchyma with  $\approx 10\mu\text{m}$  roundish cells; recognizable by its warty surface and colour, although two other warty types were encountered that had very similar anatomy but somewhat different surface colour and texture; Figures 1.10c-1.10f.

**em13 (*Lactarius deliciosus*) (Fr.) S.F.G.:** light brown with orange latex and green patches and bruising, older parts dark turquoise, surface smooth to slightly felty with age,

occasional bright-green round rhizomorphs with inner hyaline vessel-like hyphae, outer mantle a net synenchyma of 2-3  $\mu\text{m}$  hyphae; usually easily recognized by the green colour and orange latex, but when the green colour is lacking examine the mantle closely for laticiferous hyphae yellow to orange in colour; Figures 1.5a, 1.5b.

**em14:** light brown to dark brown, rough with dark bumps or warts; mantle surface synenchymatous with 13  $\mu\text{m}$  triangular, thick-walled and often blackish cystidia; cells of outer mantle roundish 15-25  $\mu\text{m}$  in diameter; recognizable at 40X by its rough sandpaper-like surface, confirmed by distinctive cystidia; Figures 1.5c, 1.10g, 1.10h.

**em15:** light brown, finely woolly, old tips grey, outer mantle a felt prosenchyma of 3  $\mu\text{m}$ , smooth hyphae with both clamped and unclamped hyphae; although only encountered once, the woolly surface was unique, more obviously textured than em65.

**em19:** white, grainy surface, net prosenchyma surface over a synenchyma of roundish cells; hyphae smooth, wiggly, 3  $\mu\text{m}$ , clampless; somewhat distinctive thin white surface texture; the only type with white colour, smooth clampless hyphae and a synenchymatous mantle of roundish cells; Figures 1.11a, 1.11b.

**em20 (*Amphinema byssoides* (Pers.: Fr.) Erikss.):** brownish yellow to white where mantle traps air, brown elsewhere; rhizomorphs abundant, brownish yellow, running parallel to the root; hyphae of outer mantle and emanating hyphae 2-4  $\mu\text{m}$ , smooth, clamped; one of only three distinctly yellow types, recognizable by its colour and rhizomorphs; Figure 1.5d.

**em21:** light orangish brown, smooth matt, irregularly pinnate, abundant emanating hyphae form sheets, emanating hyphae 3  $\mu\text{m}$ , smooth with rounded clamps, mantle surface a regular synenchyma of cells 10x15  $\mu\text{m}$ ; em21 and em22 were the only types found to form sheets of mycelium in the soil and are similar morphologically except that em 22 has a slightly textured surface and occasional loose rhizomorphs: em21 and em22 are recognizable by these sheets, irregular ramification and tortuous tips; em21 is distinguished by its smooth mat surface and regular synenchyma; Figures 1.11c, 1.11d.

**em22:** light yellow to yellowish brown, tips bent to tortuous, mantle surface stringy to finely felty, abundant extramatrical mycelium that renders soil cohesive or forms sheets; hyphae in mantle and emanating hyphae smooth, 3-4  $\mu\text{m}$  and with rounded clamps; outer mantle a felt prosenchyma; similar in appearance to em21; Figure 1.11e.

**em25:** light brownish orange, finely grainy, almost smooth matt, tips bent to tortuous, outer mantle an interlocking synenchyma of cells 5x16  $\mu\text{m}$ ; similar in morphology to em21, em56 and em69, but with distinct mantle anatomy; Figure 1.11f.

**em27:** white, reticulate, tips long and with many gentle bends; abundant, loosely organized rhizomorphs; hyphae clamped, 4  $\mu\text{m}$ , finely to heavily ornamented with roundish bumps; tentatively distinguished from types 2, 11, 16, 49, 58, 60, 67 and 68 by uniformly white mantle with only very small flecks of cortex showing through, appearance of rhizomorphs, and shape of the tips; distinguished anatomically by presence of clamps and type of ornamentation; Figure 1.11g, 1.11h, 1.11a.

**em28**("Fagirhiza spinulosa"): dark brown, short-spiny surface texture, outer mantle a regular synenchyma of cells 14x17  $\mu\text{m}$ ; abundant cystidia, 30-100  $\mu\text{m}$  long, 10  $\mu\text{m}$  wide at base and 1-2  $\mu\text{m}$  wide at tip, bulbous at base and with a short to long, narrow, curved distal portion; immediately recognizable as the only dark, short-spiny type; Figures 1.12b-1.12d.

**em29**: bright, slightly greenish yellow, grainy appearance; occasional round rhizomorphs with 10  $\mu\text{m}$  vessel-like central hyphae; outer mantle a net prosenchyma or synenchyma of 5  $\mu\text{m}$  clamped hyphae; in mineral soil only; immediately recognizable by its bright yellow colour and paucity of rhizomorphs; Figures 1.6a, 1.6b.

**em31**: light brown, may have extensive white areas due to cystidia, long-spiny or fuzzy: outer mantle synenchymatous with rounded or polygonal cells 15 (7-30)  $\mu\text{m}$  wide, mantle thin; cystidia 3-4 septate, 100 (60-200)  $\mu\text{m}$  long, 3-6  $\mu\text{m}$  wide, hyaline, curved, with basal cell like other cells of mantle; recognizable at 40X by its fuzzy surface of curved cystidia; similar to em17, which has longer (300 $\mu\text{m}$ ), straighter, less abundant rhizomorphs; Figures 1.12e, 1.12f.

**em42**: brown, grainy texture, abundant emanating hyphae give the surface a fuzzy appearance; outer mantle a synenchyma with large (21x37), roundish, thick-walled (2-3  $\mu\text{m}$ ) cells; cystidia sharp, triangular to knife-shaped, 10-40  $\mu\text{m}$  long, 6-25  $\mu\text{m}$  wide, walls 1-3  $\mu\text{m}$  thick; emanating hyphae 8  $\mu\text{m}$ , unclamped, smooth, thick walled (2  $\mu\text{m}$ ); not distinctive at 40X, similar mantle and cystidia to those of em14, but em 42 has larger hyphae emanating and in the inner mantle, no black colouration in the mantle and a less rough surface texture; Figures 1.12g, 1.12h.

**em43:** brown, smooth shiny, abundant white cottony emanating hyphae; mantle thin, a single cell layer of interlocking synenchyma or a net prosenchyma or synenchyma over an inner mantle layer of interlocking synenchyma; interlocking synenchyma not as interlocking as some, intermediate between a net and interlocking synenchyma; emanating hyphae 3-5  $\mu\text{m}$  with bulbous clamps and globular deposits up to 1  $\mu\text{m}$  diameter; easily recognizable by smooth shiny mantle and white cottony emanating hyphae; Figures 1.13a, 1.13b.

**em46:** light pinkish-white, reticulate; rhizomorphs abundant, less than 140  $\mu\text{m}$ ; outer mantle a net prosenchyma of 4-5  $\mu\text{m}$  wide, clamped, smooth to finely ornamented hyphae; yellow under 254 nm UV light, bright yellow under 366 nm UV light, rhizomorphs blue in KOH; recognizable by pink colour and stringy rhizomorphs; Figures 1.6c, 1.13c.

**em57 (*Lactarius*):** yellowish-brown with white thread-like laticiferous hyphae; outer mantle an interlocking synenchyma with 2-3  $\mu\text{m}$  wide, thick-walled (1-1.5  $\mu\text{m}$ ) hyphae, laticiferous hyphae 6  $\mu\text{m}$  wide; distinguished from other *Lactarius* species by cell size and mantle type; Figures 1.13d, 1.13e.

**em59:** white with occasional round rhizomorphs, tips with several smooth bends, granular surface; outer mantle an interlocking synenchyma of 4  $\mu\text{m}$  cells with a clear surface layer that appears broken into plates, some surface hyphae highly branched; rhizomorph surface like that of mantle, with purplish 2  $\mu\text{m}$  wide hyphae, 12  $\mu\text{m}$  wide vessel-like hyphae inside; emanating hyphae purplish, 3  $\mu\text{m}$ , highly ramified, with many hyphal tips, not clamped; tips turn blue-green in Meltzer's reagent and reddish-purple in sulfovanillin; similar in appearance to types 5, 19, 52 and

54, distinguished by surface plates and colour reaction in Meltzer's; Figures 1.13f, 1.13g.

**em62** (*Russula aeruginea* Lindblad: Fr.): brown, white in places where cystidia trap air, surface grainy to fuzzy to short-spiny on account of cystidia; outer mantle a net prosenchyma of 4  $\mu\text{m}$ , clampless, smooth hyphae; cystidia 25  $\mu\text{m}$  long by 4  $\mu\text{m}$  wide with a spore-like bulge at the tip; surface texture recognizable, type confirmed by unique cystidia; Figure 1.13h.

**em65**: very light brown with lemon yellow tints in places, felty or finely woolly; outer mantle a net prosenchyma of 3-4.5  $\mu\text{m}$  wide, smooth, clamped hyphae, mantle thick; recognized by the felty mantle with yellow colour in places; Figure 1.6d.

## Appendix 2A. Description of sites

Unless otherwise stated, methods of site description were as specified by Luttmerding et al. (1990). Two sites were studied, both on southeastern Vancouver Island (Fig. 2A.1), in the Very Dry Maritime subzone of the Coastal Western Hemlock biogeoclimatic zone (CWHxm) (Green and Klinka 1994) (Table 2A.1). Each plot has a well-drained Brunisolic soil (Tables 2A.2-2A.5), moderate slope and a southerly exposure (Table 2A.6). Soil descriptions were based on a single soil pit per plot, with the exception that the numerical measurements for the Koksilah soil were based on four pits per plot. Soil textures were measured by the Bouyoucos hydrometer method (Bouyoucos 1962). A 60 m square plot was studied in each of the two old-growth and two mature stands. Douglas-fir, *Pseudotsuga menziesii*, dominated each stand. Western hemlock, *Tsuga heterophylla* and western red cedar, *Thuja plicata*, accounted for less than 20% of the dominant or co-dominant trees in each of the four plots. Stand characteristics for each plot are reported in Table 2A.7 and additional details of stand structure at Goldstream are presented in Figure 2A.2. The mature stands are 76% and 68% as tall (maximum height) as old growth at Koksilah and Goldstream respectively and trees are 39% and 51% as large (maximum diameter at breast-height) (Table 2A.7). At each site there was a patchy distribution of abundant suppressed Douglas-fir (Table 2A.8, Fig. 2A.2), especially in the old-growth plots. *Gaultheria shallon* (salal) dominates the understory vegetation in all plots (Table 2A.8).

The first site studied, several kilometres north of the Koksilah River (Fig. 2A.1), is in the western (moister) variant (CWHxm2) of the biogeoclimatic subzone (Table 2A.1). The second site studied is in the eastern variant (CWHxm1), near the outlet of Goldstream Lake. Despite this difference in biogeoclimatic subzone variant, vegetation indicates a

drier soil environment at Koksilah. The Goldstream site fits the "03 FdHw - Salal" site series of the CWHxm (Green and Klinka 1994). Vegetation was surveyed in May at Goldstream. The time of the survey at Koksilah was not reported. At Koksilah, *Holodiscus discolor* is a codominant understory shrub and cedar, hemlock and red huckleberry (*Vaccinium parvifolium*) and are less frequent there (Table 2A.8), indicating a drier site series, between "03 FdHw - Salal" and "02 FdPl - Cladina". The Koksilah site is near the top of a slope, is steeper and is more exposed to wind and is classified as a poor quality site (slow growth of tree height). The Goldstream site is near the bottom of a small knoll, less than a kilometre from Goldstream Creek (Fig. 2A.1.) and is classified as a medium quality site.

In each plot, types of soil substrate, LFH depths, humus types, and amounts of coarse woody debris were recorded along an equilateral triangular transect 51 m on a side. All woody debris over 1 cm diameter was measured and the decay-class determined (Sollins 1982). Seventy-five measurements of substrate and LFH depths and 12 determinations of humus type were made at regular intervals along the transects. LFH materials over mineral soil comprised 75% of the soil substrate (Fig. 2A.3). Almost all decayed wood was covered by LFH materials, and bedrock covered by LFH was present in all plots. The frequency of substrate types was very similar in old-growth and mature plots within each site. At Goldstream the forest floor (L+F+H) and the F and H layers were deeper than in the mature plot, but there were no significant differences in LFH depths at Koksilah (Fig. 2A.4). At Goldstream the humus layer in the mature plot was predominantly humifibrimor (7 of 12 samples), with some fibrihumimor (2/12), raw moder (1/12) and fibrimor (2/12); while in the old growth humus was less variable: humifibrimor (10/12), fibrihumimor (1/12) and typical moder (1/12). Humus was classified by the

degree to which organic and mineral layers were delineated and the relative thicknesses of the L, F and H layers. Well-decayed logs over 16 cm diameter made up 95% of the volume of coarse woody debris (Fig 2A.5, 2A.6). At each site about twice as much coarse woody debris was present in old-growth as in mature plots. Logs of 40 cm diameter or larger were common in old-growth and virtually absent from mature plots. Maximum rotted wood depths were greater in the old-growth than the mature plot at Goldstream (Fig. 2A.7).

**Tables and figures**

**Table 2A.1. Selected climatic parameters for the biogeoclimatic subzone variants containing the Koksilah and Goldstream sites (Klinka *et al.* 1991)**

<b>Biogeoclimatic zone, subzone, variant</b>	<b>CWHxm1 (Goldstream)</b>	<b>CWHxm2 (Koksilah)</b>
<b>mean annual precipitation (mm)</b>	<b>1,425.0</b>	<b>1,969.0</b>
<b>mean precipitation April-September (mm)</b>	<b>346.0</b>	<b>462.0</b>
<b>mean precipitation of driest month (mm)</b>	<b>37.0</b>	<b>49.0</b>
<b>mean precipitation of wettest month (mm)</b>	<b>238.0</b>	<b>322.0</b>
<b>mean annual temperature (C)</b>	<b>9.4</b>	<b>8.8</b>
<b>mean temperature of coldest month (C)</b>	<b>1.8</b>	<b>1.4</b>
<b>mean temperature of warmest month (C)</b>	<b>17.0</b>	<b>16.5</b>
<b>months with mean temperature &gt; 10C</b>	<b>5.4</b>	<b>5.2</b>
<b>CWHxm = Coastal Western Hemlock biogeoclimatic zone, Very Dry Maritime subzone</b> <b>CWHxm1 = eastern variant of CWHxm</b> <b>CWHxm2 = western variant of CWHxm</b>		

**Table 2A.2. Description of the soil horizons at the mature (87 yr) plot at Koksilah (Trofymow et al. 1996)**

horizon	depth (cm)	thickness (cm)	pH	Fe+Al (%)	colour	charcoal	structure	texture	fine roots	medium roots	coarse roots	
LFH	3-0	2-3	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Ah	0-4	4-5	5.05	0.585	brown 7.5YR 5/6	+	sub-angular blocky	silt loam	-	+++	-	
B	4-38	26-40	4.97	0.500	red 2.5YR 4/8	-	single grained	silt loam	-	+++	+	
BC	38-88	41-61	4.89	0.565	red 2.5YR 4/8	-	single grained	silt loam	-	+++	+	
C	88+	bedrock									NA = not assessed	

**Table 2A.3. Description of soil horizons at the old-growth (288 yr) plot at Koksilah (Trofymow et al. 1996)**

horizon	depth (cm)	thickness (cm)	pH	Fe+Al (%)	colour	charcoal	structure	texture	fine roots	medium roots	coarse roots
LFH	4-0	3-4	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ah	0-4	3-4	4.93	0.623	NA	-	sub-angular blocky	silt loam	+++	+	-
B	4-28	20-28	4.80	0.577	yellowish red 5.0YR 5/8	-	granular	silt loam	-	+++	-
BC	28-82	46-62	4.86	0.498	yellowish red 5.0YR 5/8	-	single grained	silt loam	-	+++	+
C	82+	NA = not assessed					compact basal till		-	-	-

**Table 2A.4. Description of the soil horizons at the mature (89 yr) plot at Goldstream**

<b>horizon</b>	<b>depth (cm)</b>	<b>thickness (cm)</b>	<b>texture</b>	<b>structure</b>	<b>colour</b>	<b>charcoal</b>
<b>L</b>	0-1.5	1.5-2	NA	NA	NA	-
<b>F</b>	1.5-4	1.5-2.5	NA	NA	NA	-
<b>B</b>	4-54	40-60	sandy loam	weak single grains	red 7.5 YR 5/6	-
<b>BC</b>	54-64	5-22	sandy loam	weak single grains	yellow 10 YR 6/6	-
<b>C</b>	64-	NA	sandy loam	weak single grains	yellow 2.5 Y 6/4	-

**Table 2A.5. Description of the soil horizons at the old-growth (441 yr) plot at Goldstream**

horizon	depth (cm)	thickness (cm)	texture	structure	colour	charcoal
<b>L</b>	0-.5	0-.5	NA	NA	NA	-
<b>F</b>	.5-1	0-.5	NA	NA	NA	-
<b>H</b>	1.0-3.5	2-4	silt loam	weak single grains	yellow 10 YR 6/4	-
<b>B</b>	3.5-29.5	25-27	silt loam	weak single grains	red 9 YR 6/6	+
<b>Bf</b>	29.5-47.5	17-19	silt loam	weak single grains	red 9 YR 5/6	-
<b>BC</b>	47.5-	?	sandy loam	weak single grains	yellow 10 YR 6/4	-

Table 2A.6. Comparison of sampling points in old-growth and mature stands. Summary statistics are means, standard deviations (s), sample sizes (n) and probability values for the null hypotheses of no difference between age classes.

parameter	Koksilah site					Goldstream site			
	age class	mean	s	n	p. value	mean	s	n	p. value
height of salal (cm)	mature	32	32	36	>0.5	73	47	24	<.001
	old growth	32	24	36		84	40	24	
slope (degrees)	mature	17.2	8.8	36	<.001	13.5	7.0	24	<.001
	old growth	13.2	11.3	36		8.4	6.1	24	
aspect (degrees)	mature	194	34	31	<.001	163	32	22	<.001
	old growth	133	44	29		214	44	18	
distance to nearest dominant or codominant tree (cm)	mature	77	57	36	<.001	134	114	23	<.001
	old growth	207	153	36		233	182	24	
diameter of nearest dominant or codominant tree (cm)	mature	19.0	7.1	35	<.001	32.2	13.7	23	<.001
	old growth	56	21.0	36		72.9	21.8	23	

Table 2A.7. Mensurational data (and standard errors) from mature and old-growth plots. In each subplot at Koksilah, all trees greater than 3.0 m high were measured (Blackwell and Trofymow 1993). In each subplot at Goldstream, all trees greater than 7.5 cm diameter at breast height (1.3 m) (dbh) were measured.

Site	Plot	Life-form	Species	n	Density (ha <sup>-1</sup> )	Basal Area (m <sup>2</sup> /ha)	Mean DBH (cm)	Mean Height (m)	Max. Height (m)	Mean Age (yrs)
KOK	MA	tree	Fd	79	3353	63.0 (0.0)	13.7 (0.8)	12.8 (0.6)	27.3	87 (2)
		snag	Fd	35	1485	4.6 (0.0)	5.8 (0.4)	7.3 (0.7)	22.9	n.b. n=8 for all age data
	OG	tree	Fd	44	467	87.2 (0.5)	41.7 (3.8)	21.4 (1.7)	35.9	
			Hw	1	11	0.5	24.5 (15.4)	15.4	15.4	
			all	45	478	87.7				
	snag	Fd	5	53	4.0 (0.0)	26.9 (7.6)	12.4 (3.9)	26.1		
	GOLD	MA	tree	Fd	19	807	56.5	28.0 (2.5)	24.2 (1.1)	32.2
Cr				4	170	4.4	15.6 (5.3)	11.4 (2.5)	17.8	
Hw				3	127	3.2	17.1 (3.7)	15.3 (3.3)	18.7	
all				26	1104	64.1	24.8 (2.2)	21.2 (1.4)	32.2	89 (1)
snag			Fd	9	382	5.3	12.9 (1.1)	12.3 (1.3)	19.3	
			Hw	1	42	2.2	25.5	23.2	23.2	
			all	10	425	7.4	14.2 (1.6)	13.4 (1.6)	23.2	
OG		tree	Fd	127	1101	74.4	19.4 (2.1)	13.9 (0.8)	47.0	
			Hw	2	16	0.5	18.3 (6.5)	13.3 (1.1)	14.5	
			Cr	1	8	0.1	14.3	10.5	10.5	
			all	130	1035	75.0	19.3 (2.1)	13.9 (0.8)	47.0	441 (3)
		snag	Fd	2	16	2.4	40.6 (17.7)	17.8 (2.0)	19.8	

Key:

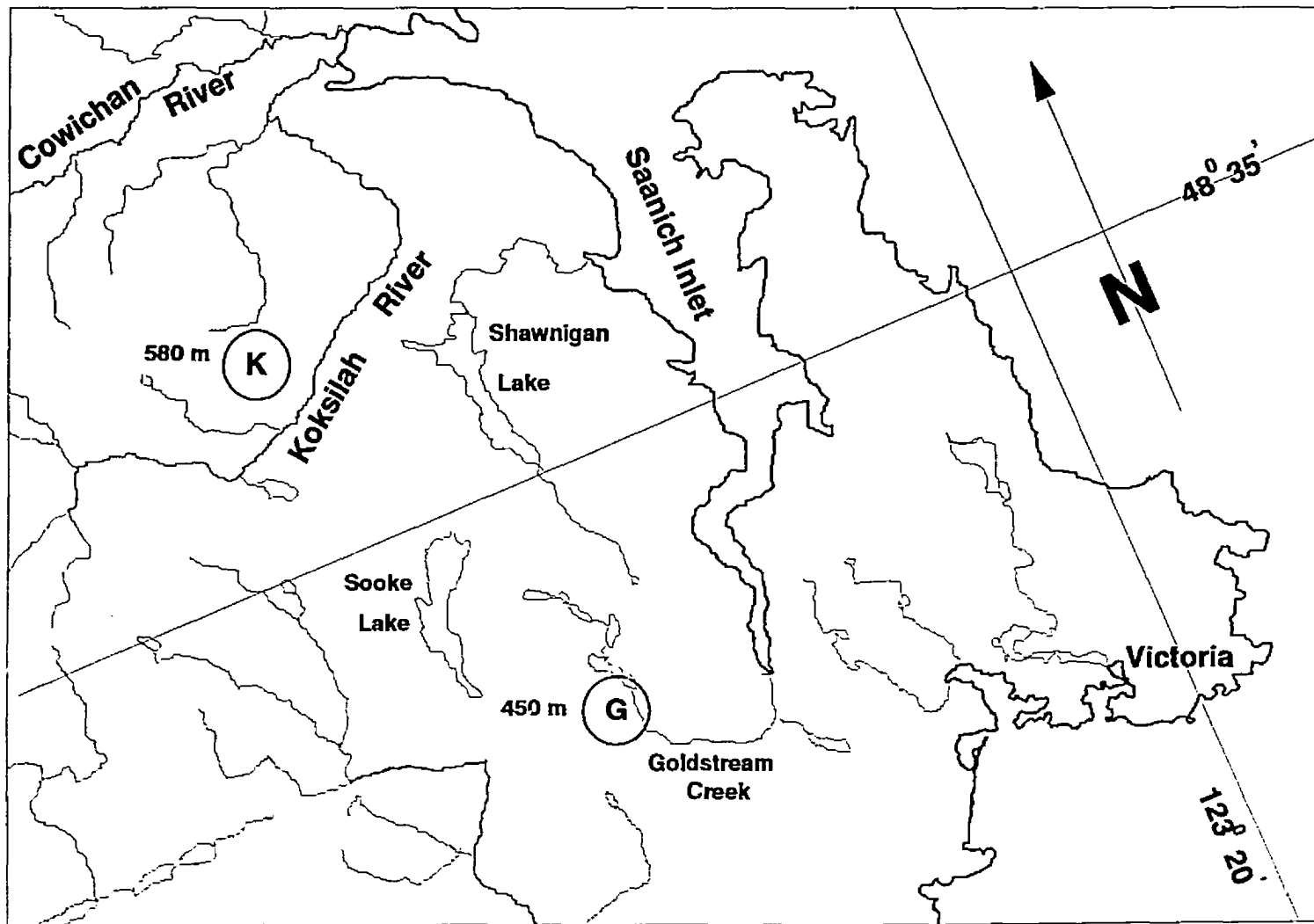
DBH=diameter at breast-height (1.3 m); KOK=Koksilah; GOLD=Goldstream; MA=mature; OG=old growth; Fd=Douglas-fir; Hw=western hemlock; Cr=western red cedar

Table 2A.8. Percent of area covered by plant species in four subplots in each of the four study plots. Tall shrubs are by definition 2-10 m tall. Data for Koksilah collected by Ryan and Frazer (Trofymow et al. 1996).

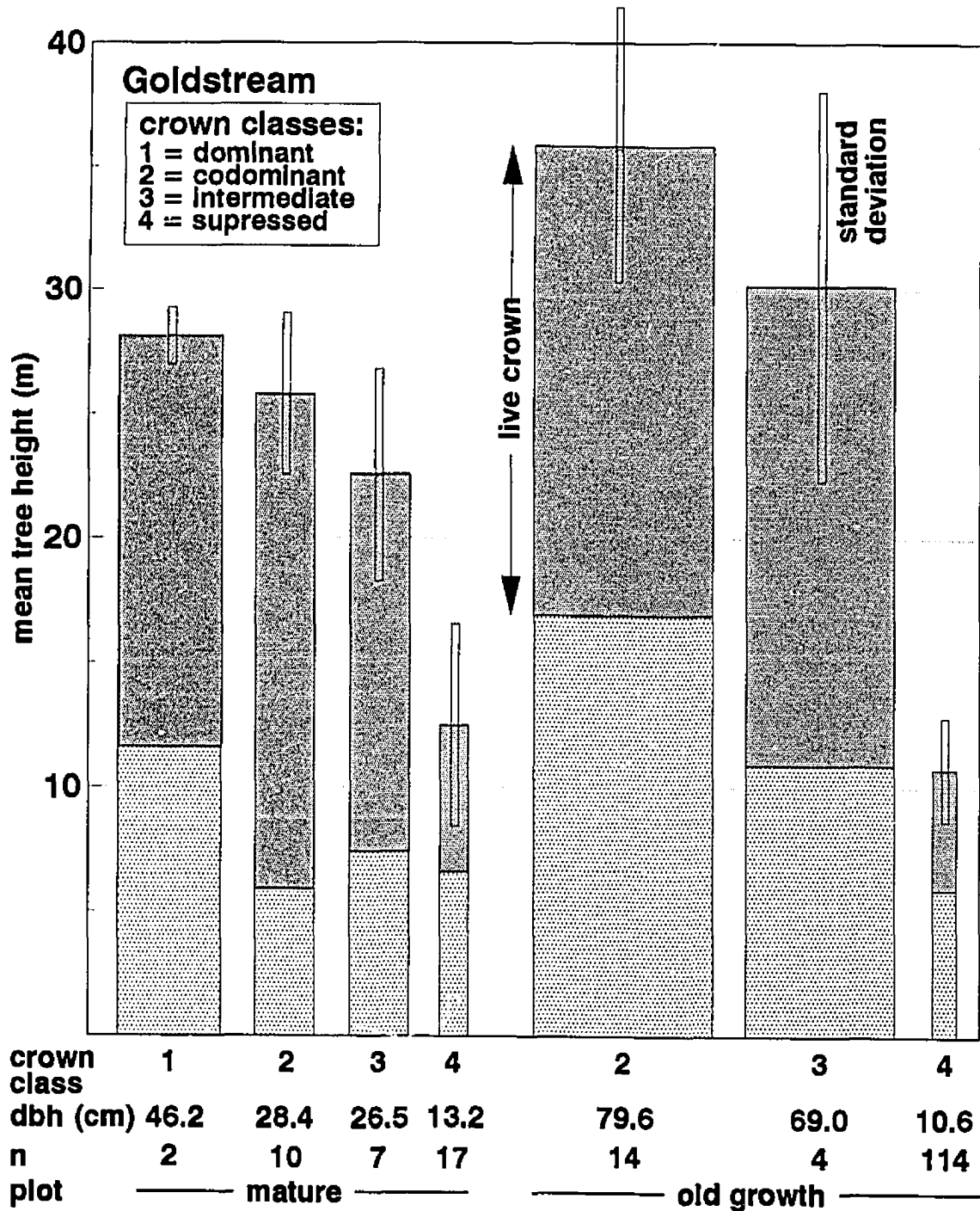
layer	species	Koksilah mature	Koksilah old growth	Goldstream mature	Goldstream old growth
dominant and codominant trees	<i>Pseudotsuga menziesii</i> (Mirb.) Franco	34	65	70	55
	<i>Tsuga heterophylla</i> (Raf.) Sarg.	0	0	1	0
intermediate or suppressed trees	<i>Pseudotsuga menziesii</i>	35	14	7	64
	<i>Thuja plicata</i> Donn ex D. Donn	1	0	30	0.05
	<i>Tsuga heterophylla</i>	0	1	30	2
	<i>Pinus monticola</i> Dougl.	0	0	0	0.1
tall shrubs	<i>Pseudotsuga menziesii</i>	10	5	0.5	0
	<i>Salix scouleriana</i> Barratt	2	0	0	0
	<i>Thuja plicata</i>	1	0	0.1	0
	<i>Holodiscus discolor</i> (Pursh) Maxim.	1	5	0	0
	<i>Tsuga heterophylla</i>	0.5	0	4	0
	<i>Gaultheria shallon</i> Pursh	0	0	30	68
	<i>Pinus monticola</i>	0	0	0.07	0
	<i>Vaccinium parvifolium</i> Sm.	0	0	0.05	0
low shrubs	<i>Gaultheria shallon</i>	35	70	31	20
	<i>Berberis nervosa</i> Pursh.	0	7	4	15
	<i>Rosa gymnocarpa</i> Nutt.	0	3	0	0.2
	<i>Thuja plicata</i>	1	0	0.07	0.8
	<i>Ribes lacustre</i> (Pers.) Poir.	0	1	0	0
	<i>Chimaphila umbellata</i> (L.) Barton	0	0.5	0.2	0.1
	<i>Holodiscus discolor</i>	0.5	0	0	0

layer	species	Koksilah mature	Koksilah old growth	Goldstream mature	Goldstream old growth
low shrubs (cont.)	<i>Tsuga heterophylla</i>	0.5	0	0.2	0
	<i>Vaccinium parvifolium</i>	0.5	0	0.05	0.08
	<i>Polystichum munitum</i> (Kaulfuss) Presl	0	0	0	0.02
herbs	<i>Moehringia macrophylla</i> Hook.	0	2	0	0
	<i>Festuca occidentalis</i> Hook.	0	1	0	0
	<i>Festuca subulata</i> Trin.	0	1	0	0
	<i>Campanula scouleri</i> Hook.	0	0.5	0	0
	<i>Collomia heterophylla</i> Hook.	0	0.5	0	0
	<i>Linnaea borealis</i> L.	0	0.5	0.2	2
	<i>Listera cordata</i> (L.) R.Br.	0	0	0.07	0.02
	<i>Pteridium aquilinum</i> (L.) Kuhn in Decken	0	0	0.02	0
	<i>Pyrola asarifolia</i> Michx.	0	0	0.02	0
	<i>Viola</i> L.	0	0	0.02	0.02
mosses and liverworts	<i>Kindbergia oregana</i> (Sull.) Ochyra	50	7	30	35
	<i>Hylocomium splendens</i> (Hedw.) B.S.G.	5	15	10	0.02
	<i>Dicranum fuscescens</i> Turn.	1	0	-	-
	<i>Dicranum scoparium</i> Hedw.	1	0	-	-
	<i>Dicranum</i> Hedw.	2	0	0.05	0.7
	<i>Isothecium myosuroides</i> Bridel	1	0	0	0
	<i>Mnium spinulosum</i> B.S.G.	0.5	0	0	0
<i>Rhacomitrium canescens</i> (Hedw.) Bridel	0.5	0	0	0	

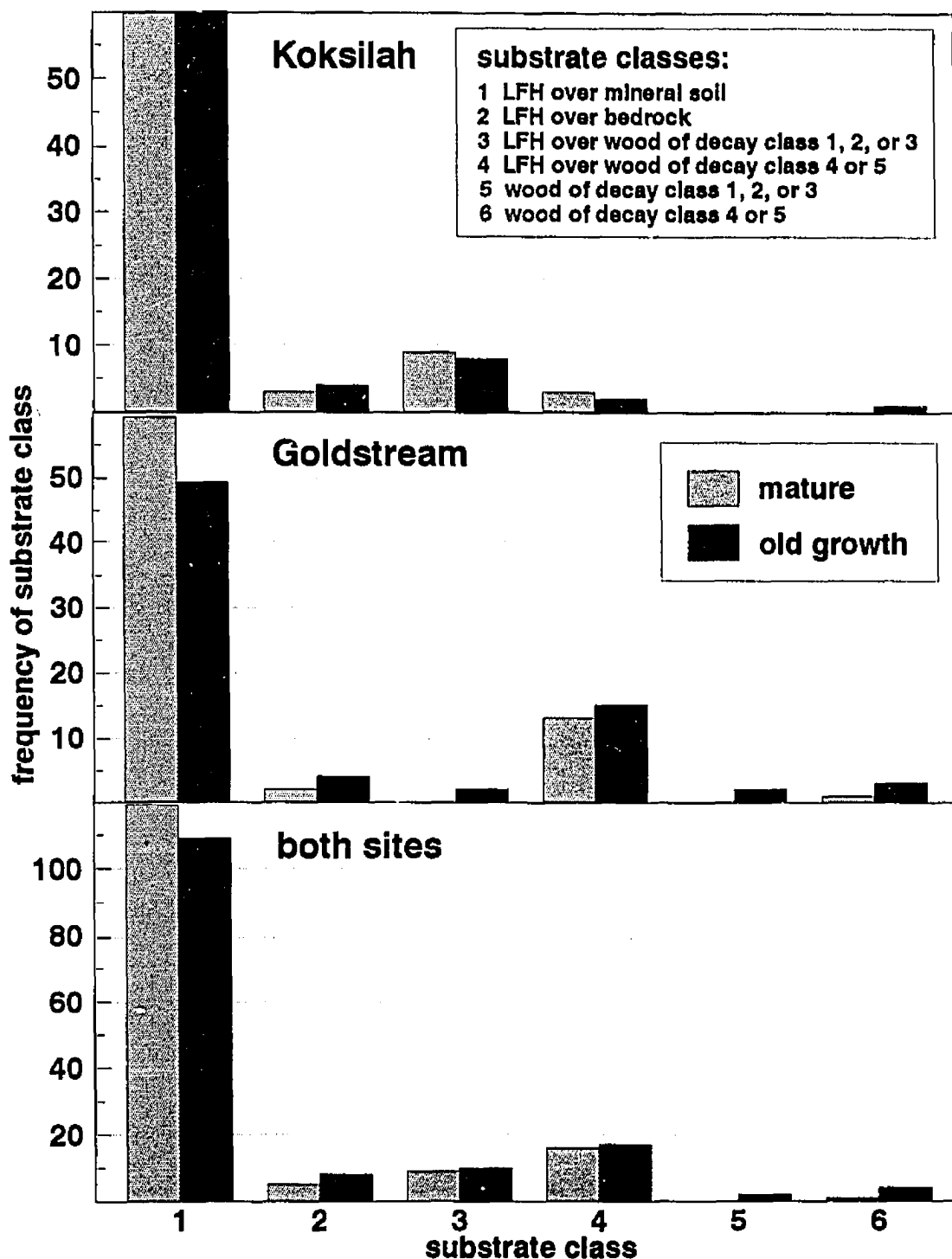
layer	species	Koksilah mature	Koksilah old growth	Goldstream mature	Goldstream old growth
mosses and liverworts (cont.)	<i>Rhytidiopsis robusta</i> (Hedw.) Broth	0.5	0	0.05	0
	<i>Trachybryum megaptilum</i> (Sull.) Schof.	0.5	2	0	0
	<i>Rhytidiadelphus loreus</i> (Hedw.) Warnst.	0	0	0.3	0
	<i>Rhytidiadelphus triquetrus</i> (Hedw.) Warnst.	0	0	0.1	0.05
	<i>Scapania bolanderi</i> Aust.	0	0	0.05	0.1
	<i>Hypnum circinale</i> Hook.	0	0	0.02	0.02
	<i>Dicranowesia cirrata</i> (Hedw.) Lindb.	0	0	0	0.05



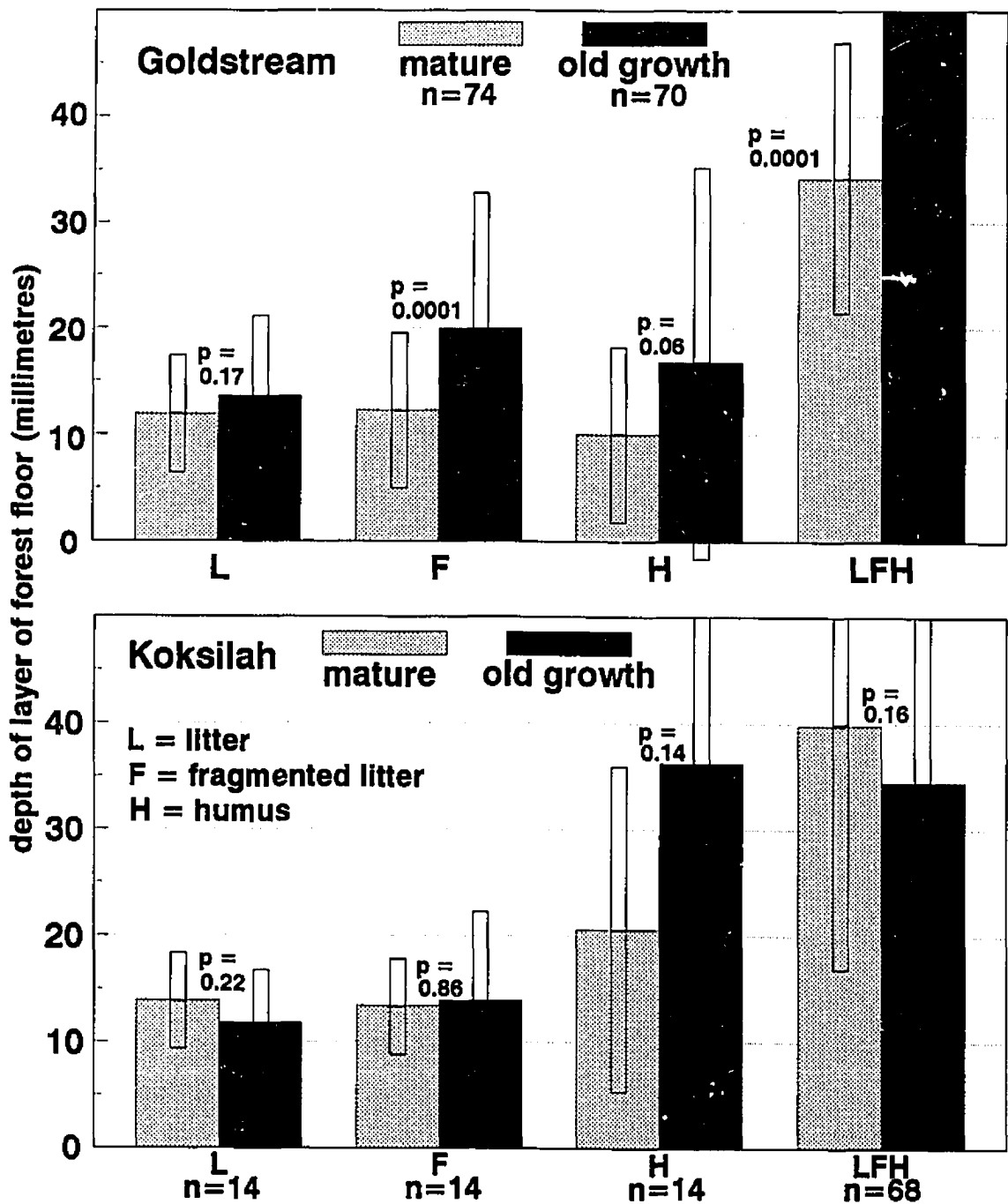
**Figure 2A.1. Location of Koksilah (K) and Goldstream (G) study sites**



**Figure 2A.2. Heights and diameters of trees in 300 sq. m. of mature forest and 1200 sq. m. of old-growth forest at Goldstream. Width of bars is proportional to tree diameter.**



**Figure 2A.3.** Amount of substrate types in mature and old-growth plots. Frequencies are numbers of observations per plot (75 total). Koksilah data by Blackwell (1992).



**Figure 2A.4.** Depths of L, F, and H layers in each plot at each site (means and standard deviations). Probability values test age-class comparisons. Depths were measured along transects (n=68-74) and at random points of mycorrhizal sampling (n=14). Koksilah LFH data by Blackwell (1992).

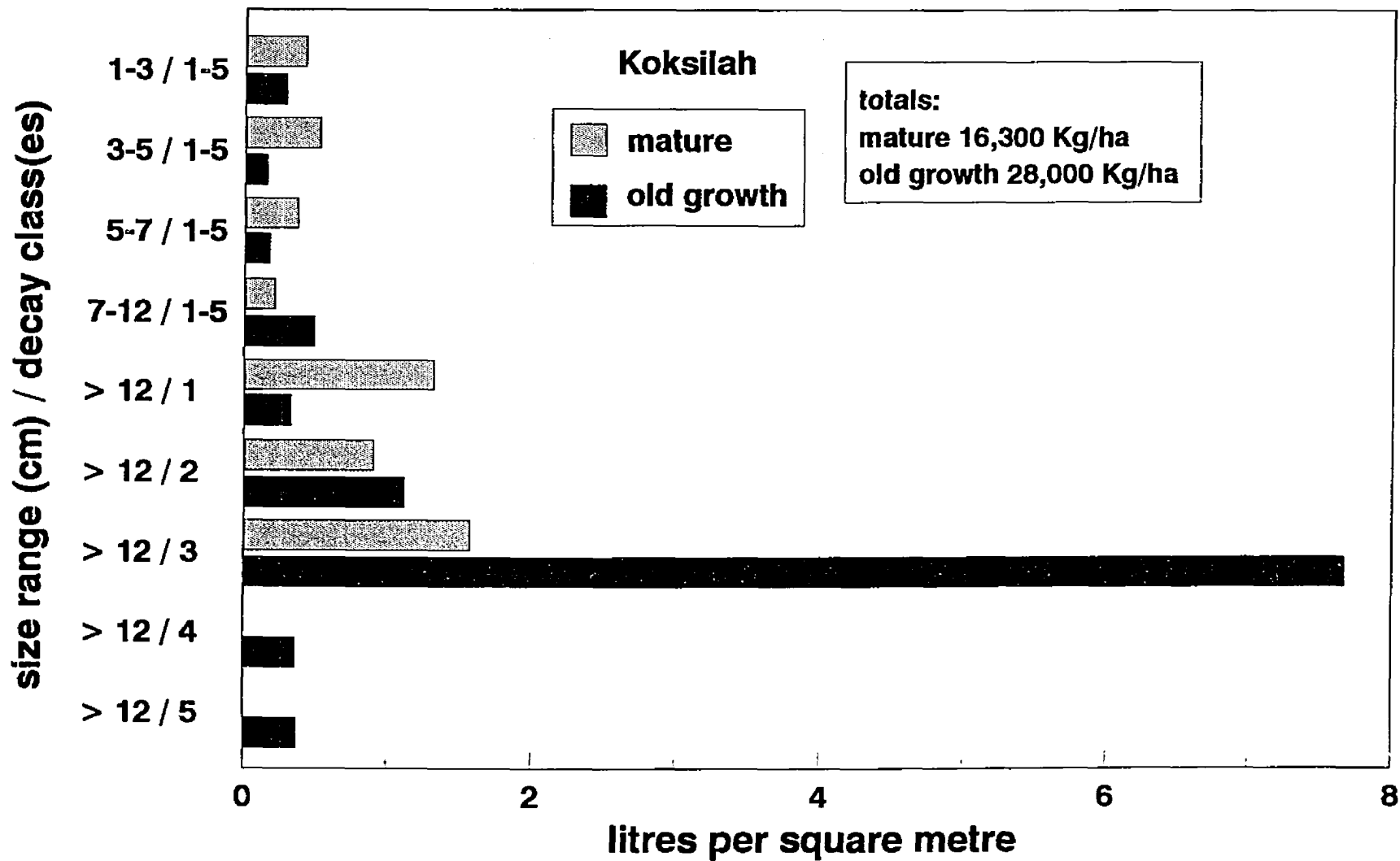


Figure 2A.5. Distribution of coarse woody debris among size and decay classes at Koksilah. Degree of decay increases with decay class (Trofymow, unpublished).

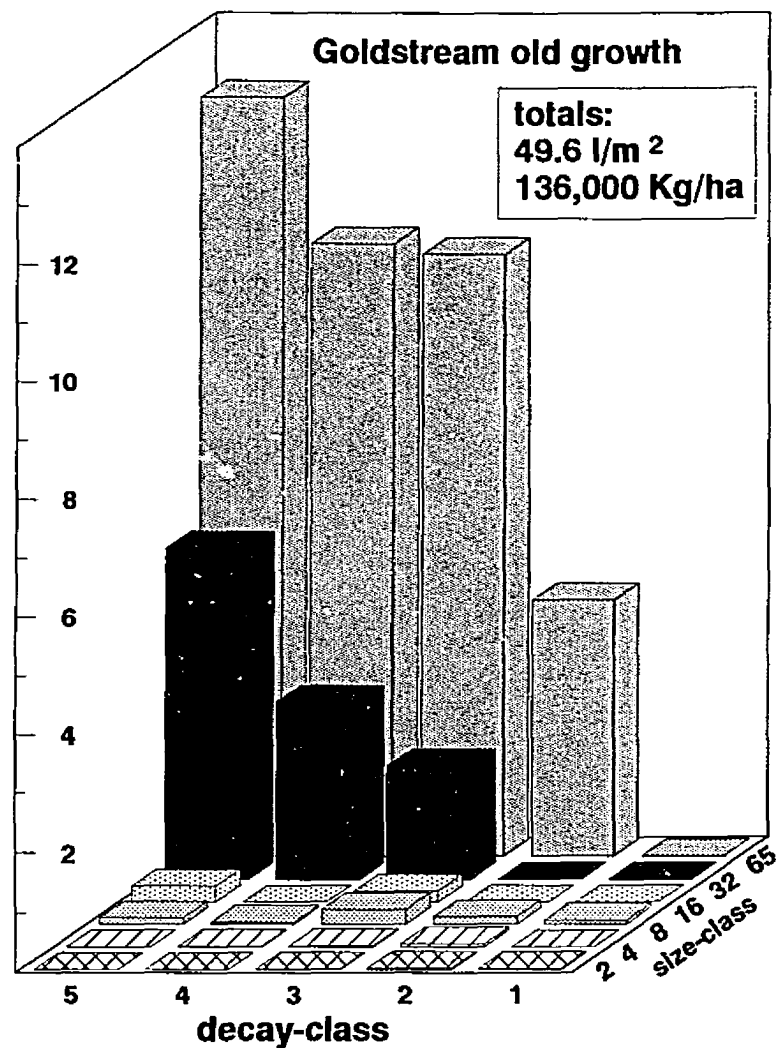
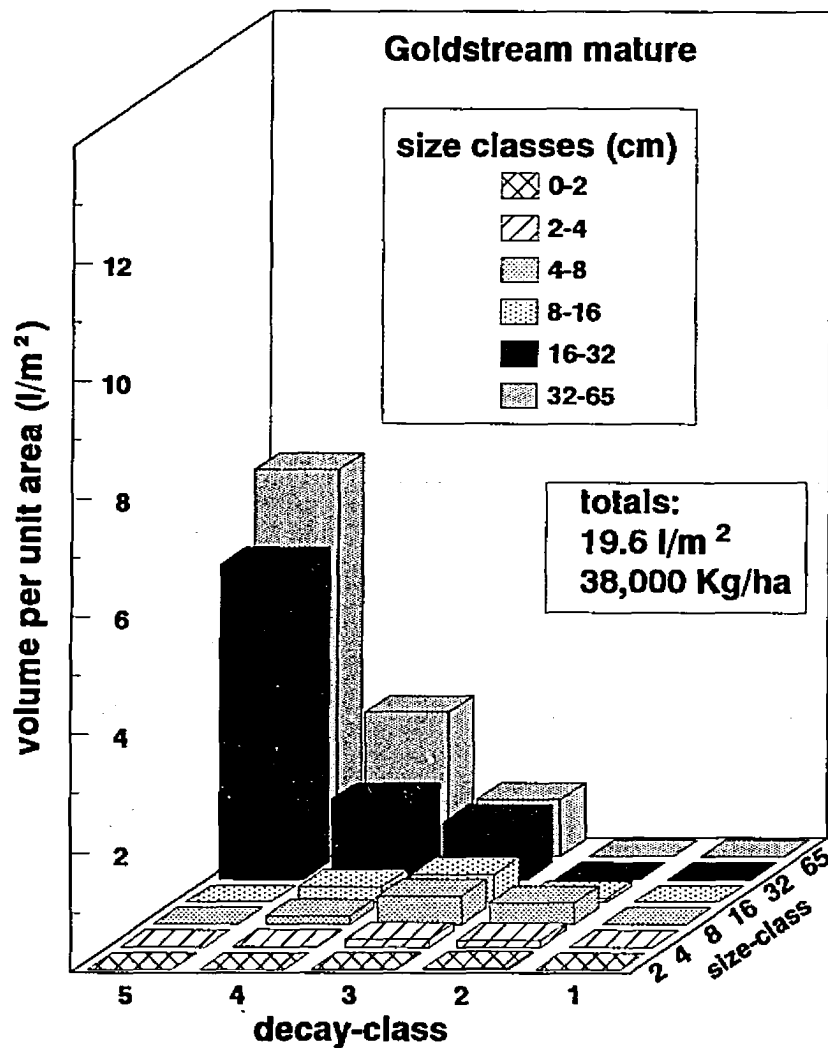
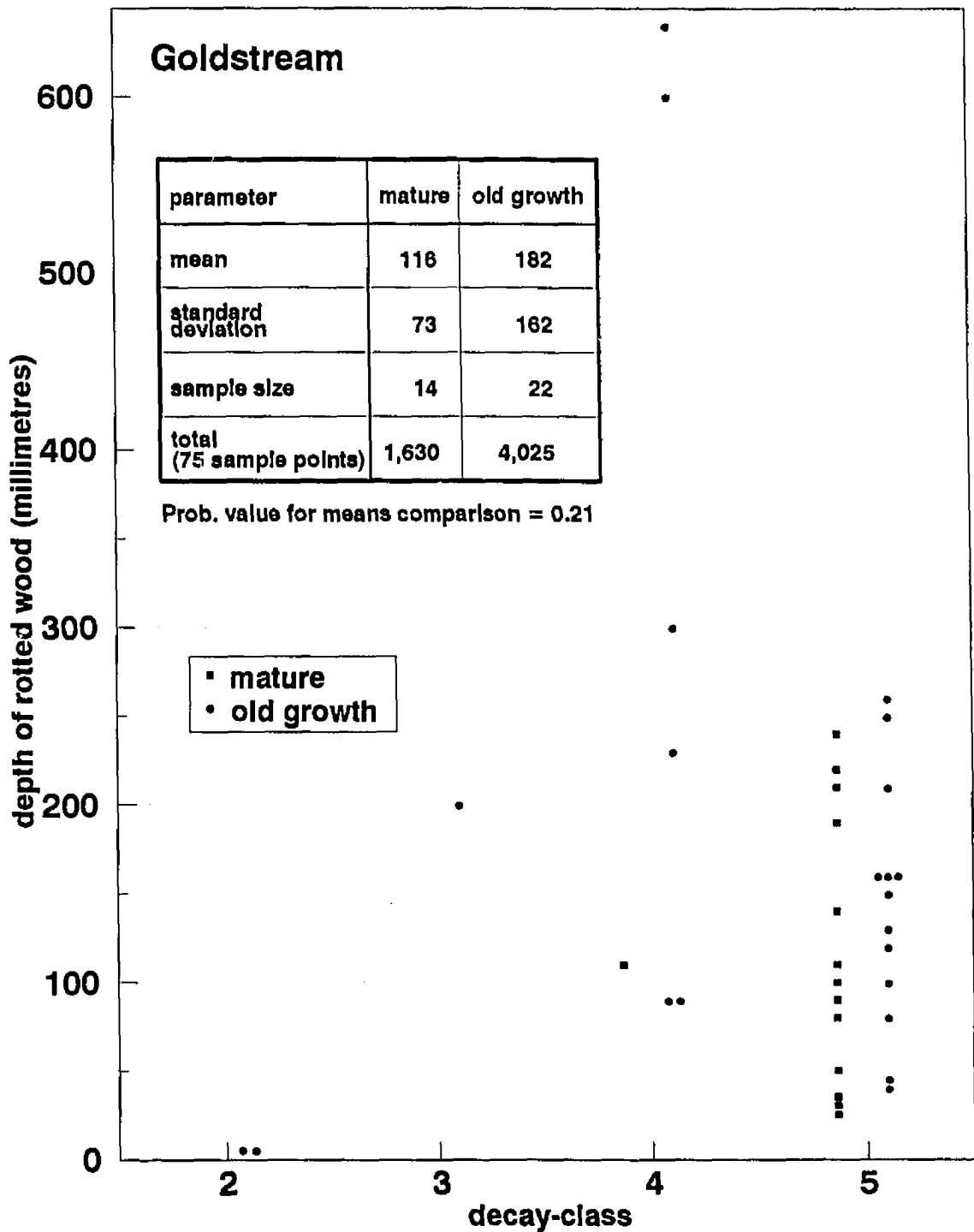


Figure 2A.6. Distribution of coarse woody debris among decay and size classes in mature and old-growth plots at Goldstream. Degree of decay increases with decay class number.



**Figure 2A.7. Depths of rotted wood in the mature and old-growth stands at Goldstream. Degree of decay increases with class number.**

**Appendix 3A. Comparisons of mean nutrient concentrations and other parameters of several soil habitats**

Tables 3A.1-3 report the results of analyses of variance of data collected at each point where soil was sampled for the survey of ectomycorrhizas.

**Tables**

**Table 3A.1. Analysis of variance for comparison of mean nutrient concentrations in soil habitats**

factor	df	pH		carbon (%)		extractable phosphorous (µg/g)		nitrogen (%)		mineralizable nitrogen (µg/g)		moisture (%)	
		F	P	F	P	F	P	F	P	F	P	F	P
habitat	6	68.4	0.0001	273	0.0001	13.5	0.001	49.6	0.0001	17.5	0.0001	53.5	0.0001
age	1	0.08	0.78	0.72	0.40	0.26	0.61	0.33	0.57	1.00	0.32	0.03	0.87
site	1	2.96	0.09	5.96	0.02	0.95	0.33	3.29	0.07	7.66	0.006	0.22	0.63
habitat * age	6	2.41	0.03	1.55	0.17	0.56	0.76	1.08	0.38	0.26	0.96	0.91	0.49
habitat * site	5	1.06	0.39	1.79	0.12	0.76	0.58	1.32	0.26	1.33	0.25	1.09	0.37
age * site	1	0.01	0.90	2.31	0.13	11.9	0.007	2.38	0.12	0.32	0.58	2.75	0.10
habitat * age * site	5	0.87	0.46	2.18	0.06	3.21	0.01	0.51	0.77	0.56	0.73	0.84	0.52

**Table 3A.2. Analysis of variance for comparison of mean thicknesses of L, F and H layers in soil habitats**

factor	df	litter		fragmented litter		humus		depth of core samples	
		F	P	F	P	F	P	F	P
habitat	4	4.54	0.002	9.30	0.0001	39.6	0.001	413.4	0.0001
age	1	0.71	0.40	5.53	0.02	7.28	0.01	1.21	0.27
site	1	0.05	0.82	15.4	0.0001	28.2	0.0001	0.38	0.54
habitat * age	4	4.50	0.00	3.76	0.01	5.82	0.0002	1.27	0.28
habitat * site	3	3.12	0.03	1.97	0.12	3.81	0.01	2.30	0.08
age * site	1	10.5	0.001	1.83	0.18	11.3	0.001	0.13	0.72
habitat * age * site	3	0.73	0.54	1.17	0.32	4.29	0.01	1.08	0.36

factor	df	types		root tips		root tips of <i>Cenococcum</i> <i>geophilum</i> (em 1)		root tips of <i>Hysterangium</i> (em2)	
		F	P	F	P	F	P	F	P
habitat	6	6.37	0.0001	3.84	0.001	6.74	0.0001	2.67	0.02
age	1	0.00	0.96	0.26	0.61	3.37	0.07	1.78	0.18
site	1	11.3	0.001	8.83	0.003	3.05	0.08	0.14	0.71
habitat * age	6	1.23	0.29	1.79	0.10	1.90	0.08	1.03	0.41
habitat * site	5	2.35	0.04	2.18	0.06	0.84	0.53	0.56	0.73
age* site	1	0.27	0.60	0.03	0.86	1.67	0.20	0.21	0.64
habitat * age* site	5	1.86	0.10	2.35	0.04	0.96	0.44	2.99	0.01