NERC Identification of ectomycorrhizas

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Institute of Terrestrial Ecology
Natural Environment Research Council

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K Ingleby, P A Mason, F T Last and L V Fleming

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1. Introduction

Until recently, it has been difficult to identify ectomycorrhizas using published descriptions, which mostly consist of brief pen-pictures of the gross morphological features and some indication of mantle structure. Microscopic evidence, when illustrated, has concentrated on cross-sectional features, following the methods of Dominik (1969). This information has frequently been insufficient to enable different ectomycorrhizas to be identified.

Chilvers (1968) produced descriptions of eucalypt ectomycorrhizas which included characters observed in whole root mounts. His evidence was concentrated on the organisation of the mantle tissue as seen in plan view and the features of associated hyphae and strands. This technique has been developed and used by us when assessing mycorrhizal populations on inoculated seedlings, following outplanting to field sites. We have found that plan views of the mantle structure provide a more comprehensive and diagnostically useful picture of different mycorrhizal fungi than evidence obtained from cross-sections. In addition, mycorrhizas can rapidly be examined using a whole root mount for quantitative as well as qualitative assessments.

Like Chilvers, Agerer (1986) and Haug and Oberwinkler (1987) have used plan views of the mantle to characterise ectomycorrhizas.However, while they concentrated on mycorrhizal associations found in mature forests, we have concentrated on those associated with young trees – our interests have been complementary.

Many of the fungi associated with mycorrhizas described in this booklet have been examined on more than one host. Mycorrhizas formed by the same fungus with different tree species were found to be broadly similar – in other words, their structure, when examined microscopically, seems to be largely host-independent. Thus, for each mycorrhizal fungus, a single description is presented, based on one particular host. The observations, therefore, support those of Godbout and Fortin (1985) who, on the basis of existing knowledge, concluded that only one description is needed for ectomycorrhizas produced by each fungus.

2. Scope and presentation

This booklet describes a rapid but accurate method for examining and characterising ectomycorrhizas.

There follows a series of 24 descriptions of ectomycorrhizas most commonly encountered by us on young trees in Britain. These have been arranged in approximate order of succession, ie early numbers appear most commonly on seedlings 1–2 years of age, whereas later numbers appear on trees 5–10 years of age.

The descriptions are presented in a spiral-bound booklet in order to facilitate their use in the laboratory.

The descriptions include observations of:

i. strands and associated hyphae;

ii. sclerotia;

iii. the mantle edge, emanating hyphae and specialised cells;

iv. the mantle as seen in plan view.

These features have been presented in a standard format designed to relate to the way in which whole root mounts are examined. The descriptions do not include cross-sectional information – we have found this useful only for measuring mantle depth and corroborating the layering of mantle structures.

It is hoped that these descriptions will:

i. facilitate communication between research workers concerned with mycorrhizas;

ii. improve the accuracy and interpretation of experimental data; and

iii. stimulate the description of more types of mycorrhizas by research workers located elsewhere, resulting in an increase in the rate of identification of unknown types.

If readers of this booklet suspect that they know the identity of any of the unknown types we have described, we would be delighted to know.

3. General guidelines for characterisation and identification

Whether or not the identity of the causal fungus is suspected, it is essential to characterise each mycorrhizal type. Referenced samples should then be stored in an herbarium.

The methods described in this booklet enable mycorrhizal types to be characterised using standardised, yet widely available, techniques. Although additional information can be obtained using a scanning electron microscope, this information should provide a positive feedback into light microscopy so that the basis of identification will remain within the scope of the light microscopist.

Identification of a mycorrhizal type may subsequently be suggested by (i) linking mycorrhizas to fruitbodies, (ii) comparing observations with published descriptions of previously identified types, or (iii) using characters established in fruitbody taxonomy.

i. Linking mycorrhizas to fruitbodies
Mycorrhiza-to-fruitbody links may be suggested by the repeated association of a mycorrhizal type with a fruitbody, or by directly tracing mycelia from the fruitbody to the mycorrhiza. These links can be confirmed by the synthesis of near-identical mycorrhizas in controlled conditions, using a pure culture of the fungus. However, descriptions should be made only from naturally occurring mycorrhizas, as those synthesised in artificial substrates and environments may grow rapidly and possess unnaturally large amounts of extramatrical mycelium.

ii. Comparing observations with published descriptions
Comparisons with published descriptions of previously identified types may suggest a specific fungus which could be confirmed by a synthesis test. However, it is more likely that a taxonomic group will be indicated with which the mycorrhizal type can be linked. Study of the identified types in this booklet reveals that similarities can be drawn between species of the same genera (ie *Lactarius, Inocybe, Laccaria* and *Hebeloma* spp.) and also between more broadly related groups (ie Humariaceae).

iii. Applying characters used in fruitbody taxonomy
Our studies have indicated that structural and hyphal features used in fruitbody taxonomy can be applied to identify the fungus occurring in the mycorrhizal state. Thus, mantles of *Lactarius* spp. and *Leccinum* spp. have been characterised using features which relate closely to those found in the cap tissue of their respective fruitbodies. In addition, distinctive colour changes, occurring with bruising, on exposure to air, or after applying chemical reagents, may prove useful, particularly when making distinctions at the species level.

Clearly, large inputs are required from taxonomists working with higher fungi so as to maximise the number of useful diagnostic characters for identifying ectomycorrhizas.

It will be important to develop the classification of ectomycorrhizas using methods (ii) and (iii), in order to identify the numerous mycorrhizal types which seldom produce fruitbodies, or which are difficult to isolate and grow in pure culture.

4. Methods for examination

4.1 Selection of material

Care should be taken to consider the age and development of mycorrhizas. The mantles of young mycorrhizas and those found at the tip of mature mycorrhizas may be loosely formed and incompletely developed; in old mycorrhizas, the mantle surface may become compacted, or even lost with the onset of senescence. Therefore, attention should be focused on fresh, recently matured mycorrhizas.

Mycorrhizas found adjoining the base of their fungal fruitbodies may, like synthesised mycorrhizas, possess excessive amounts of extramatrical mycelium and should be avoided when making descriptions.

Mycorrhizas should be examined fresh whenever possible. The examination of material preserved in glutaraldehyde or formol acetic alcohol (FAA) is not easy as the inner layers of the mantle become obscured. This is less significant when observing mycorrhizas with reasonably thick and compacted mantles, which can be peeled from underlying cortical cells with fine forceps or dissecting needles. This technique also improves clarity for photographic purposes. However, fresh mycorrhizas are most desirable, and material can be stored in water at 4°C for up to one week.

4.2 Preparation

Mycorrhizal samples should be soaked in water overnight and then washed clean in gently running water. Roots growing in mineral soils can be cleaned readily, but those growing in soils of a more organic nature will require the careful (and tedious!) removal of adhering particles, using fine forceps under the stereo dissecting microscope.

4.3 Macroscopic separation

(×5–×50 magnification)

After cleaning, mycorrhizas are covered with water in a petri dish for examination under the stereo dissecting microscope. Populations of mycorrhizas can initially be separated on features such as colour, form, size, associated hyphae, strands and sclerotia. At the higher level of magnification (×50), individual hyphae and specialised mantle surface cells, such as setae and cystidia, may just be discernible. These features should be recorded while the mycorrhizas are still fresh.

The validity of the separation should be confirmed by selecting a minimum of five typical members of each population for microscopic examination of a whole root mount. Where there are mixtures of similar mycorrhizas, further samples should be taken. Having completed the following microscopic examination and established uniformity, and therefore confidence in the macroscopic separation, quantitative assessments of each type can be made if required.

4.4 Microscopic separation

(×500–×1000 magnification)

4.4.1 Preparation of slides

Mycorrhizas should be mounted on glass slides in both lactophenol cotton blue and toluidine blue for about 10–15 seconds, before being squashed firmly under a cover glass. Several mycorrhizas can be examined under a single cover glass.

Mounting stains used are:

i. 0.1% (w/v) cotton blue in 10% (v/v) lactophenol/H_2O

This is a good general-purpose stain to examine associated hyphae, strands and mantle surfaces, staining most fungal tissues blue. We use a small concentration of lactophenol to avoid shrinkage of stained cytoplasm, which makes details of septa and clamp-connections difficult to observe.

ii. 0.1% (w/v) aqueous toluidine blue
This stains cell walls and is effective in highlighting the structure of smooth compacted mantles. It is also useful in looser mantle structures, as it can penetrate to lower tissues without staining the surface hyphae too strongly. Because of the metachromatic properties of toluidine blue, many fungi produce a diagnostically useful, if not distinctive, colour reaction which may range from blue → purple → violet → pink.

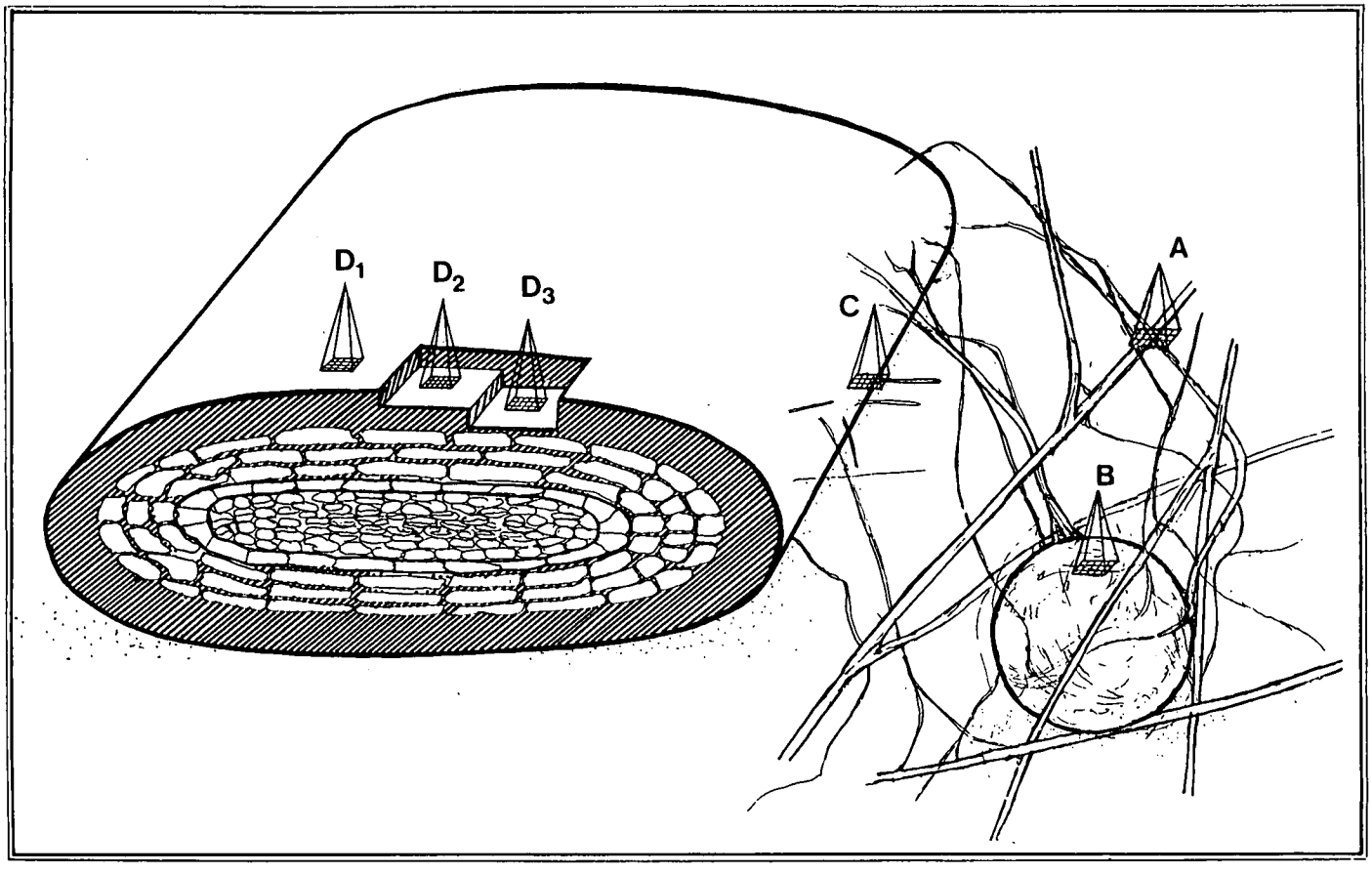
4.4.2 Features examined (Figure 1)

A. *Strands* and associated hyphae.
B. *Sclerotia.*
C. *Mantle edge,* emanating hyphae, specialised elements. These features are observed by moving to the edge of the mycorrhiza and focusing on the mantle surface, which is then viewed tangentially.
D. *Mantle* as seen in plan view. One to three layers can be distinguished in different mycorrhizas. Where there is only one distinct layer, it has been designated D1; where there are two layers, the surface is designated D1 and the inner D2; where there are three layers, the surface, intermediate and inner layers are designated D1, D2 and D3. In some rare instances, it is possible to observe the Hartig net where mantles are very thin or absent.

*NB.*Not all of these features will be found in each mycorrhizal type.

4.5 Preservation A sample of each mycorrhizal type should be preserved in 2% glutaraldehyde and stored at 4°C in an herbarium.

Figure 1. Sketch of a squashed mycorrhiza showing the location of features described in the microscopic examination



5. Using the descriptions

5.1 Text

5.1.1 Designation: class, order, family and species of the associated fungus are given, where known.

5.1.2 Associated trees: a list of tree species is given on which the mycorrhizal type has been observed. The species on which photographic plates, drawings and measurements have been made is shown in bold print.

5.1.3 Identification: the basis of identification of the mycorrhizal type is indicated using the criteria discussed in Section 3, ie synthesis, fruitbody links, literature descriptions, or fruitbody taxonomy.

5.1.4 Macroscopic appearance: the colour, where distinctive or useful, is given the reference number used in the *Flora of British fungi colour identification chart* (Royal Botanic Garden 1969).

5.1.5 Microscopic appearance: each feature outlined in Section 4.4.2 is either described or recorded as 'not observed'. Mantle tissues are described using the terminology of Chilvers (1968), who proposed two basic structures, each of two types:

i. *prosenchyma* – a loosely organised structure with abundant interhyphal spaces: *felt* or *net*
ii. *synenchyma* – a compact structure with few obvious interhyphal spaces: *irregular* or *regular.*

Although we have adopted Chilvers' subdivisions, we have found it necessary to include an additional subdivision of synenchyma, namely a *net synenchyma*, where the cells are compacted but remain distinctly elongated. The five resulting mantle types are illustrated in Figure 2.

5.1.6 Distinguishing features: comparisons are drawn with other similar or related mycorrhizal fungi, and characteristics of particular diagnostic value are emphasised. Clues to the possible identification of unknown mycorrhizas are indicated.

5.1.7 Ecology: notes on distribution and host range are recorded using both mycorrhiza and fruitbody observations.

5.2 Illustrations

5.2.1 Macroscopic: two or more colour photographs are shown, including a general view of mycorrhizas and close-up views of individual mycorrhizas or associated features.

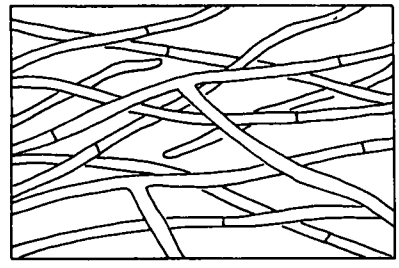
5.2.2 Microscopic: a series of black-and-white photographs show the features used in identification, and are complemented by line drawings which highlight those features. The drawings also show variations which could not be represented without the inclusion of large numbers of photographs. The line drawings were taken from tracings of photographic prints and to the same scale. A bar representing 20 µm is shown on each drawing.

The illustrations are presented in a standard format, beginning at the top of the page with strands and sclerotia, followed by emanating hyphae and the mantle edge, and finally progressing down through the different layers of the mantle, as seen in plan view. Each feature is given the standard nomenclature outlined in Section 4.4.2.

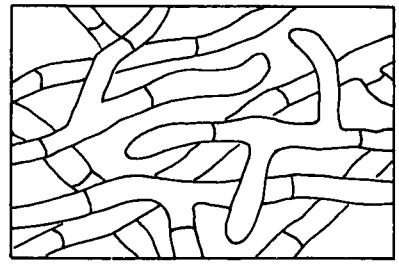
If appropriate, the transition of mantle surface characters (D1) from young to mature mycorrhizas is also shown. This transition may also be seen when moving from the tip along a mycorrhiza to its base.

Features such as thickened cell walls, hyphal encrustations, cell inclusions, etc, are shown, if they occur regularly and are of diagnostic value.

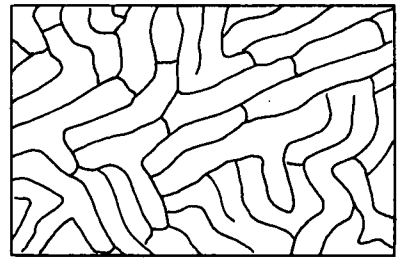
Figure 2. Terminology of the five structural mantle types used in the descriptions



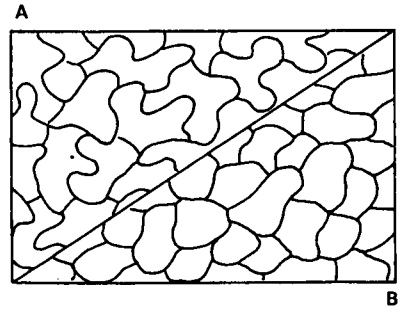
1. Felt prosenchyma
Cells distinctly elongated.
Hyphae similar to those emanating from the mantle. Not organised.



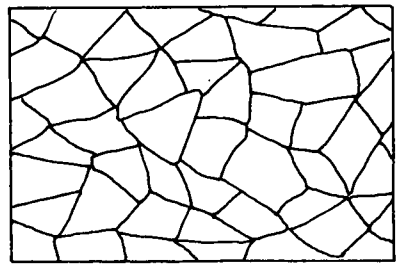
2. Net prosenchyma
Cells distinctly elongated.
Hyphae wider, shorter-celled and more branched than those emanating from the mantle. Loosely organised.



3. Net synenchyma
Cells distinctly elongated.



4. Irregular synenchyma
Cells not distinctly elongated with generally rounded walls.
A interlocking
B not interlocking



5. Regular synenchyma
Cells isodiametric with generally straight-sided walls.

6. Glossary of terms applied to mycorrhizas

Adpressed — flattened.

Clamp-connection (clamp) – a short, curved or enlarged hypha forming a bulge over the septa of many basidiomycetes.

Concolorous – of the same colour.

Cystidium – a terminal cell found on the surface of the mantle, usually of a distinctive shape (plural: cystidia).

Dichotomous – branching into two more or less equal arms.

Differentiated hypha – filamentous terminal hypha found on the surface of the mantle. These hyphae are of a determinate length and may be branched, but are not distinctly thick-walled, dark-coloured or pointed.

Emanating hypha – hypha found connected to the mantle surface.

Flexuous – hypha with undulating walls forming a filament of irregular diameter.

Globose – more or less spherical.

Isodiametric – cells of more or less uniform diameter.

Labyrinthine – intricate, entwined structure of hyphal elements.

Laticiferous – wide, dichotomously branched hypha, usually with opaque granular cytoplasm exuding a milky or colourless latex. Typically associated with species of *Lactarius*.

Pinnate – mycorrhiza with side branches arranged in two opposite rows along the main axis.

Prosenchyma – a type of mantle structure, see Section 5.1.5.

Reticulate – macroscopic appearance of a mycorrhiza produced by an irregular, reflective mantle surface.

Rind – the hard outer layer found with many sclerotia.

Sclerotium – a compact, often spherical mass of fungus (plural: sclerotia).

Septate – hypha with cross walls.

Septum – a cross wall of a hypha (plural: septa).

Seta – bristle-like hair found on the surface of the mantle, distinctly thick-walled, dark-coloured and pointed (plural: setae).

Sinuous (of a mycorrhiza or hypha) – wavy or undulating but of more or less uniform diameter.

Specialised element – terminal cell or hypha found on the mantle surface, eg seta, cystidium or differentiated hypha.

Spine – narrow, sharply pointed projection.

Strand – a linear aggregation of hyphae. Subdivided into two basic types:

i. *differentiated* – organised in two or more layers, usually with an inner core of larger-diameter hyphae
ii. *undifferentiated/simple* – composed of only one type of hypha.

Striate – mycorrhiza marked with furrows or lines.

Synenchyma – a type of mantle structure, see Section 5.1.5.

Tortuous (of a mycorrhiza or hypha) – twisted or crooked.

Verrucose – coarse or fine, wart-like encrustations of the outer hyphal wall.

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8. The descriptions

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No. 24 *Amanita muscaria*	107

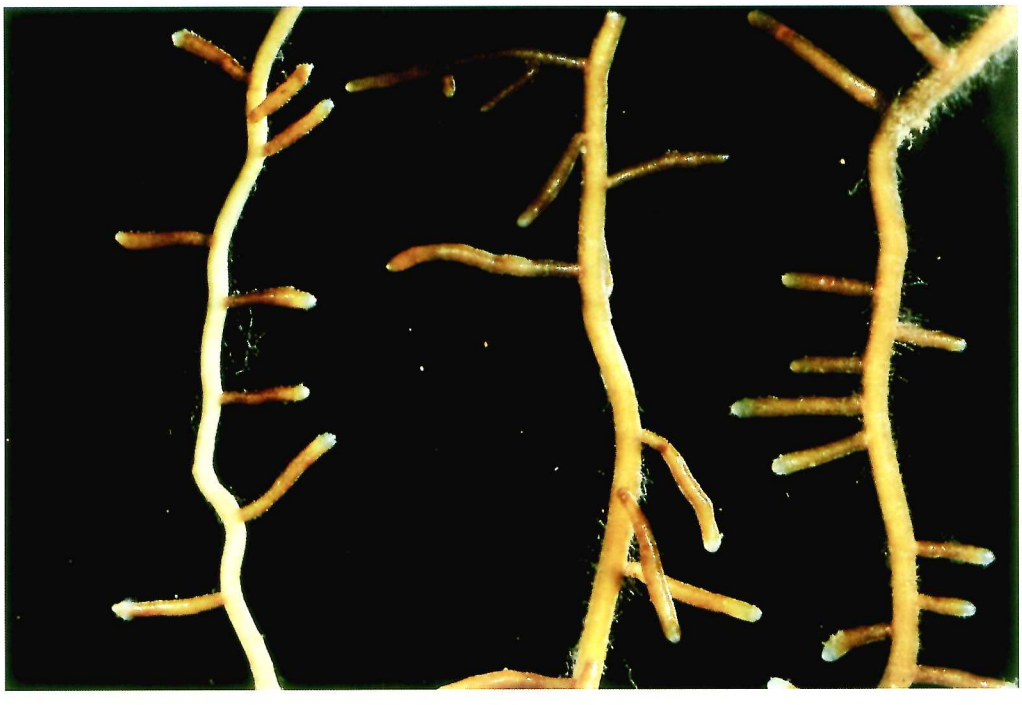
Associated trees:
Picea sitchensis

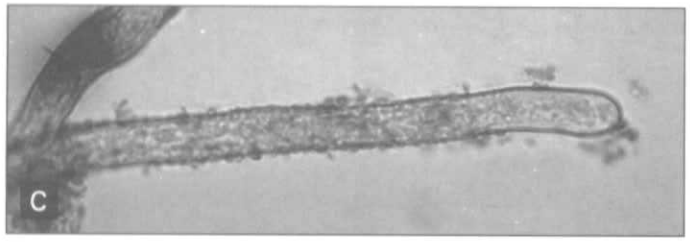
Class: **ASCOMYCOTINA**
Order: **PEZIZALES**
Family: **HUMARIACEAE**

1

Humaria hemisphaerica

(Wigg.: Fr.) Fuckel

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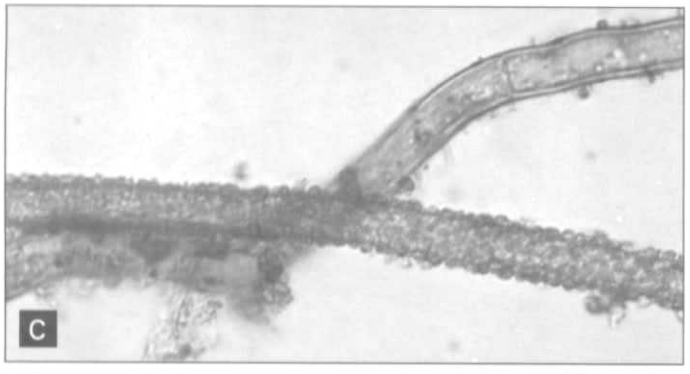
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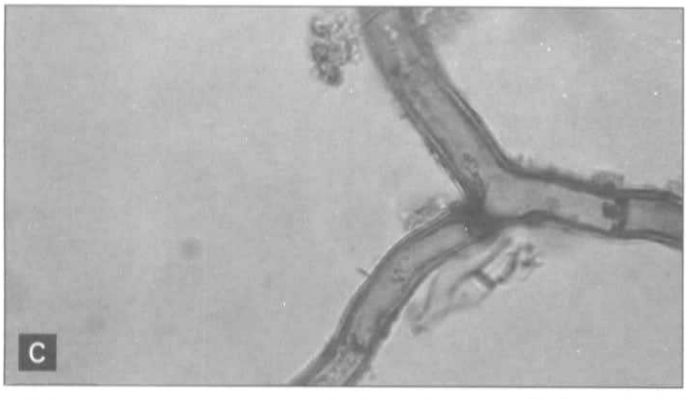
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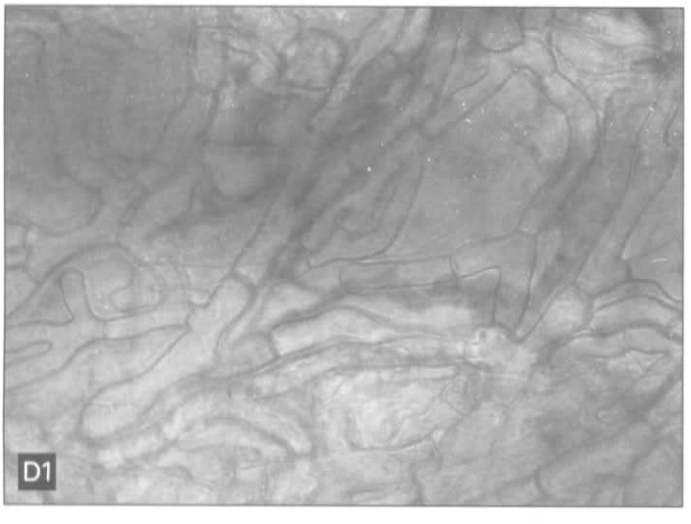
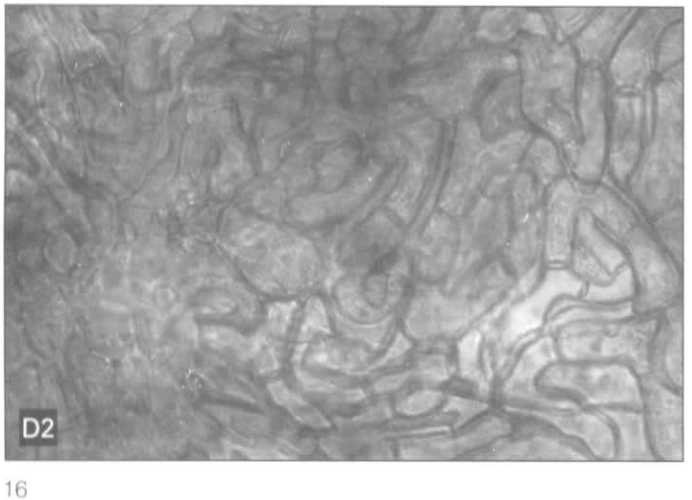

D1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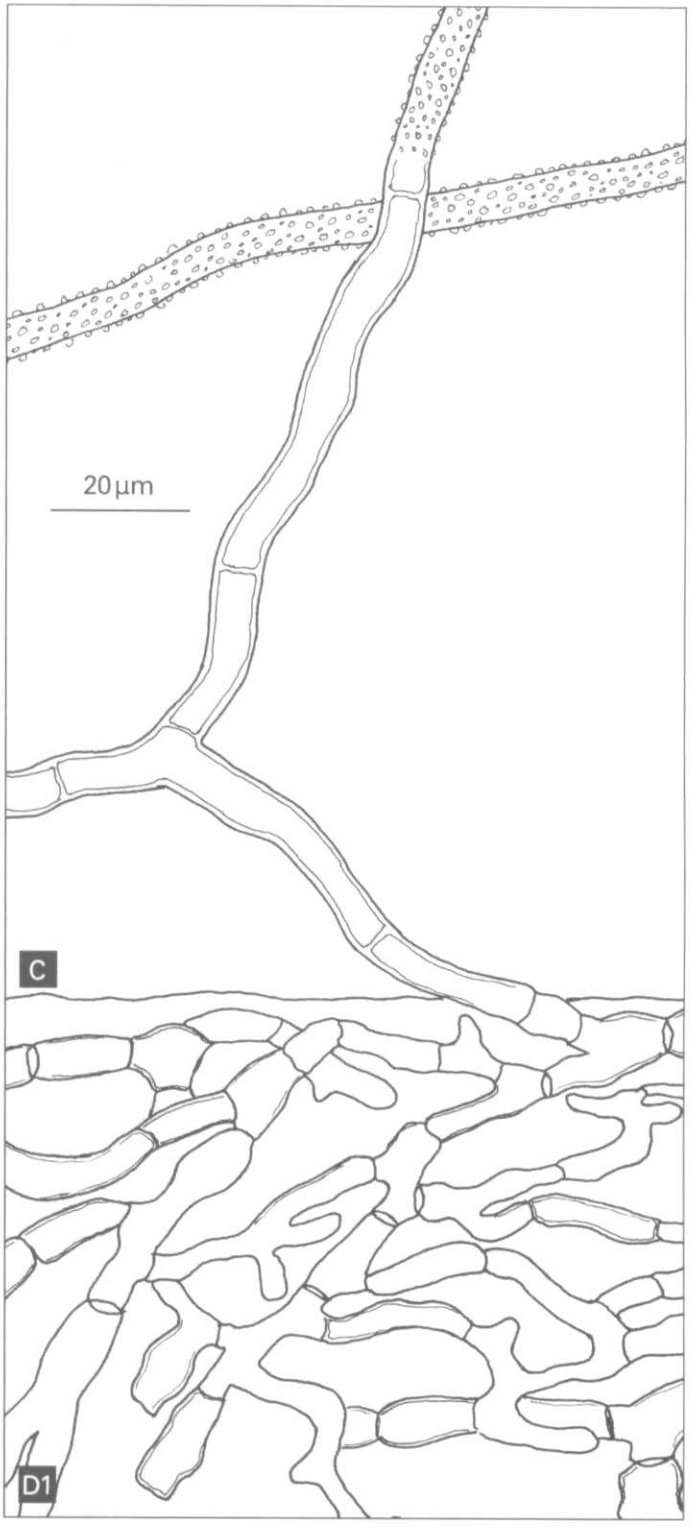
20 µm
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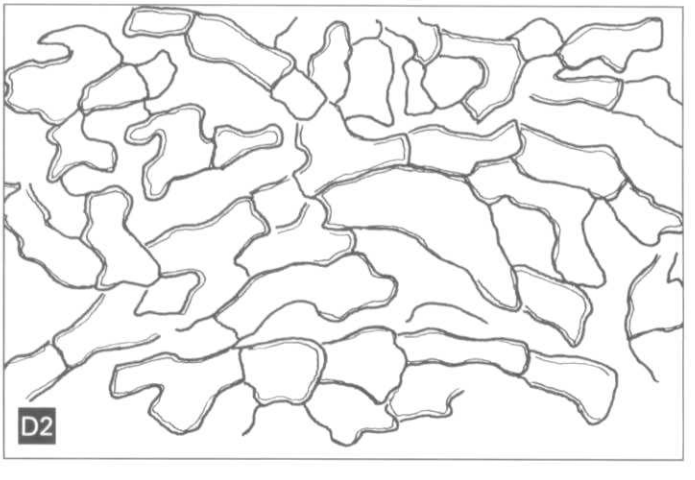
D1


D2

Humaria hemisphaerica

(Wigg.: Fr.) Fuckel

Identification:
Synthesis, fruitbody links, literature description

Macroscopic appearance

Mycorrhizas are thin, fairly straight and infrequently branched. The main axis is <7 mm in length and <0.4 mm in diameter.

The mantle surface appears reticulate and shiny. Occasionally, hyphae, readily seen with the dissecting microscope, can be found extending far from the mantle surface.

Mycorrhizas are fawn (29) when young, changing to dark brick (20) with age, and have a pale tip where the mantle is absent.

Microscopic appearance

A. *Strands:* not observed.

B. *Sclerotia:* not observed.

C. *Mantle edge:* loosely formed.
Emanating hyphae: those closest to the mantle surface are 4–8 µm in diameter, distinctly thick-walled, septate and frequently branched. These hyphae become smaller in diameter, infrequently branched and coarsely verrucose further away from the mantle.
Specialised elements: not observed.

D. *Mantle:* <10 µm in depth where present.
D1. *Surface:* a net prosenchyma of hyphae which are highly branched, with distinctly thickened walls and septa. The hyphal cells are often inflated, narrowing at the septa (4–10 µm in diameter). The mantle is often incomplete and rarely more than 2 or 3 cells in depth.

D2. *Inner:* as the surface hyphae age, or an inner mantle is formed, the hyphae become thicker-walled and shorter-celled (<20 µm in length), fusing together to form a net synenchyma.

Distinguishing features

This mycorrhiza is readily distinguished by characteristics of emanating hyphae and mantle surface as belonging to a group previously termed 'E-strain' fungi. We have described *H. hemisphaerica* and *Tricharina gilva* (Boud.) Eckblad within this group, while Danielson (1984) has also described *Sphaerosporella brunnea* (Alb. & Schw.: Fr.) Svrcek & Kubicka mycorrhizas. All belong within the family Humariaceae and are difficult to distinguish from each other. The heavily thickened mantle cell walls of *H. hemisphaerica* give the mycorrhiza its characteristic red-brown colour which distinguishes it from *T. gilva.*

Ecology

Mycorrhizas of this group are commonly associated with coniferous tree seedlings in the glasshouse and nursery, decreasing rapidly in numbers after outplanting to field sites.

Fruitbody observations suggest that this fungus is more widespread, although we have only recorded it in association with *Picea sitchensis* (Bong.) Carr.

References

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YANG, C.S. & WILCOX, H.E. 1984. An E-strain ectendomycorrhiza formed by a new species *Tricharina mikolae. Mycologia,* **76,** 674–684.

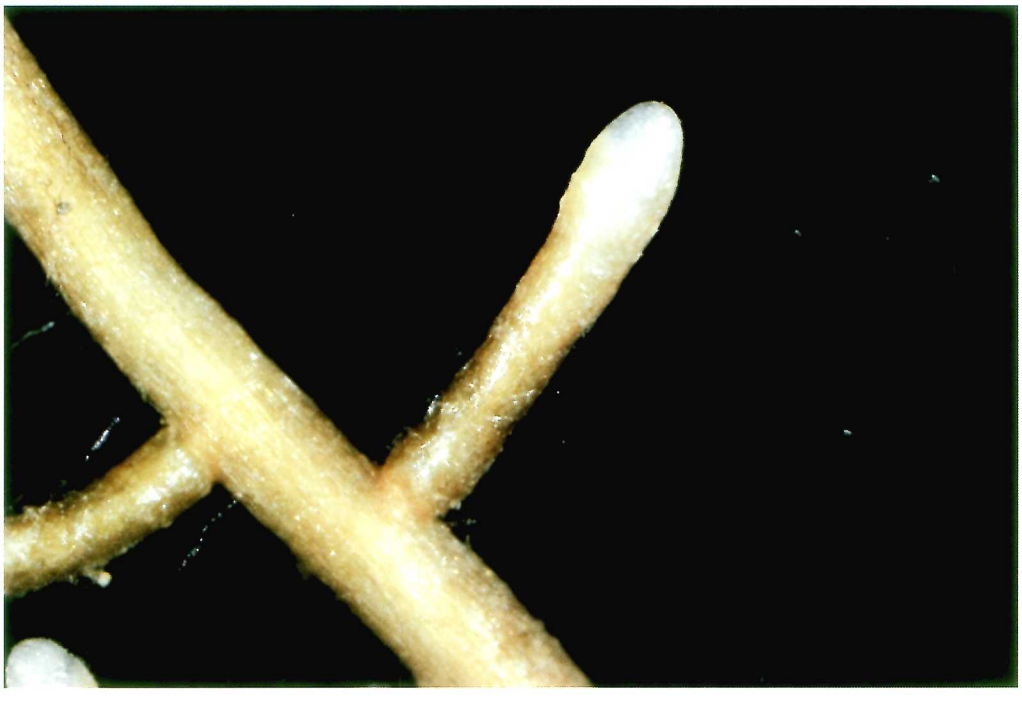
Associated trees:
Picea sitchensis
Pinus contorta
Pseudotsuga menziesii

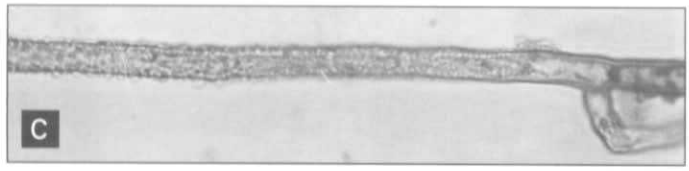
Class: **ASCOMYCOTINA**
Order: **PEZIZALES**
Family: **HUMARIACEAE**

2

Tricharina gilva

(Boud.) Eckblad

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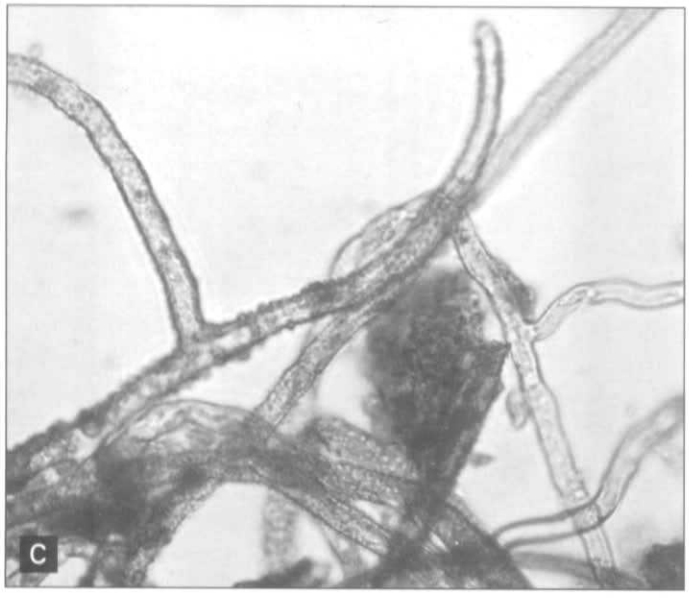
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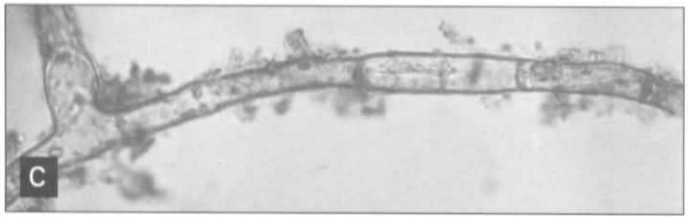
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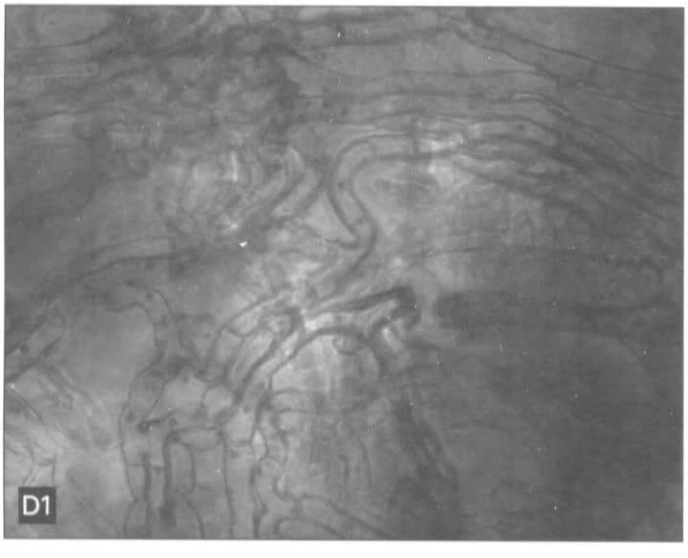
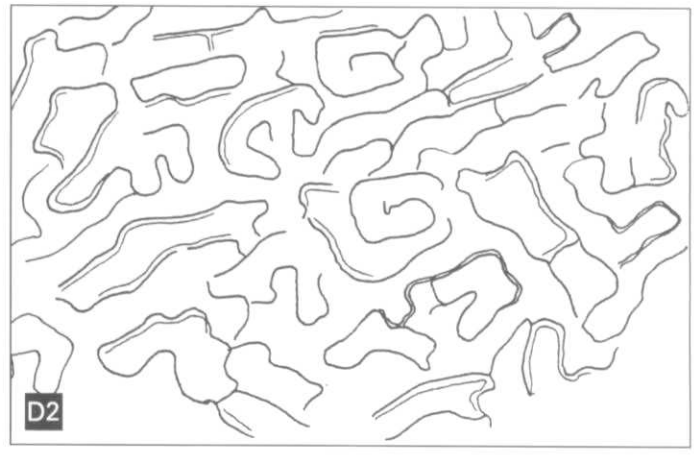

D1


D2


20 µm
C
D1


D2

Tricharina gilva

(Boud.) Eckblad

Identification:
Synthesis, fruitbody links, literature description

Macroscopic appearance

Mycorrhizas are thin, fairly straight and infrequently branched. The main axis is <9 mm in length and <0.4 µm in diameter.

Occasionally hyphae can be found extending far from the mantle surface.

Mycorrhizas are pale straw-coloured when young, changing darker brown with age.

Microscopic appearance

A. *Strands:* not observed.
B. *Sclerotia:* not observed.
C. *Mantle edge:* loosely formed.
Emanating hyphae: those closest to the mantle surface are 3–7 µm in diameter, distinctly septate and frequently branched. These hyphae become smaller in diameter, infrequently branched and coarsely verrucose further away from the mantle.
Specialised elements: not observed.
D. *Mantle:* <8 µm in depth, where present.
   D1. *Surface:* a net prosenchyma of hyphae which are highly branched and septate. The hyphal cells are often inflated, narrowing at the septa, and are 3–8 µm in diameter. The mantle is often incomplete and rarely more than 2 or 3 cells in depth.
   D2. *Inner:* has the surface hyphae age, or an inner mantle is formed, the hyphae become thicker-walled and shorter-celled (<25 µm in length), fusing together to form a net synenchyma.

Distinguishing features

This mycorrhiza is readily distinguished by characteristics of emanating hyphae and mantle surface as belonging to a group previously termed 'E-strain' fungi. We have described *T. gilva* and *Humaria hemisphaerica* (Wigg.: Fr.) Fuckel within this group, while Danielson (1984) has described *Sphaerosporella brunnea* (Alb. & Schw.: Fr.) Svrcek & Kubicka mycorrhizas. All belong within the family Humariaceae and are difficult to distinguish from each other.

Ecology

Mycorrhizas of this group are commonly associated with coniferous tree seedlings in the glasshouse and nursery, decreasing rapidly in numbers after outplanting to field sites.

Fruitbody observations suggest that this fungus may have a broader host range than *H. hemisphaerica*.

References

DANIELSON, R.M. 1982. Taxonomic affinities and criteria for identification of the common ectendomycorrhizal symbiont of pines. *Can. J. Bot.,* **60,** 7–18.

DANIELSON, R.M. 1984. Ectomycorrhiza formation by the operculate discomycete *Sphaerosporella brunnea* (Pezizales). *Mycologia,* **76,** 454–461.

DENNIS, R.W.G. 1968. *British Ascomycetes.* Stuttgart: J. Cramer.

THOMAS, G.W., ROGERS, D. & JACKSON, R.M. 1983. Changes in the mycorrhizal status of Sitka spruce following outplanting. *Pl. Soil,* **71,** 219–232.

WILSON, J., MASON, P.A., LAST, F.T., INGLEBY, K. & MUNRO, R.C. 1987. Ectomycorrhiza formation and growth of Sitka spruce seedlings on first-rotation forest sites in northern Britain. *Can. J. For. Res.,* **17,** 957–963.

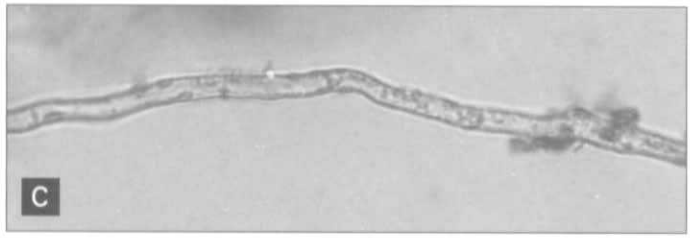
YANG, C.S. & WILCOX, H.E. 1984. An E-strain ectendomycorrhiza formed by a new species *Tricharina mikolae. Mycologia,* **76,** 674–684.

Associated trees:
Picea sitchensis

Class: **ASCOMYCOTINA**
Order:
Family:

3

Type: ITE.1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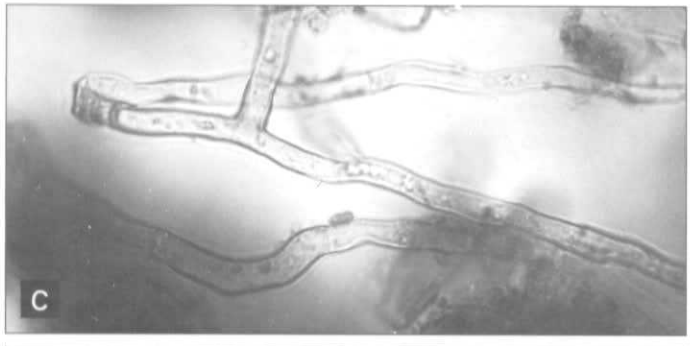
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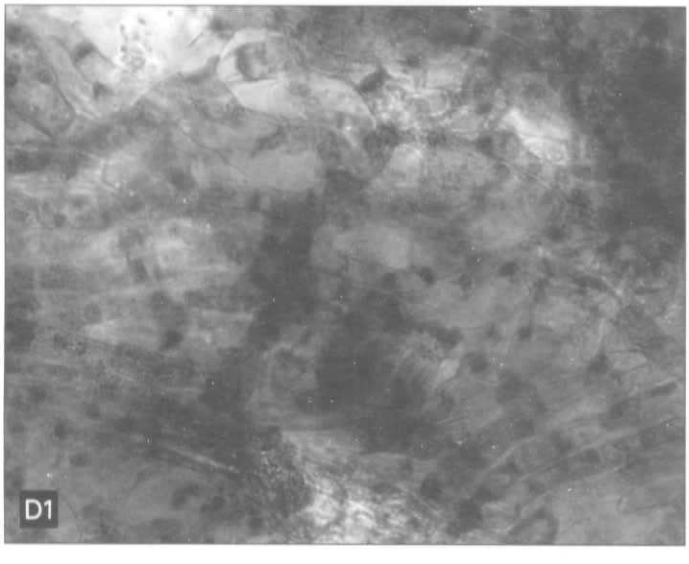
C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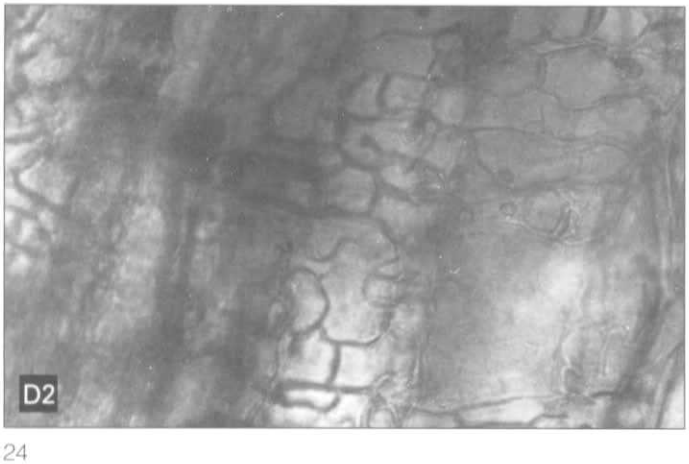
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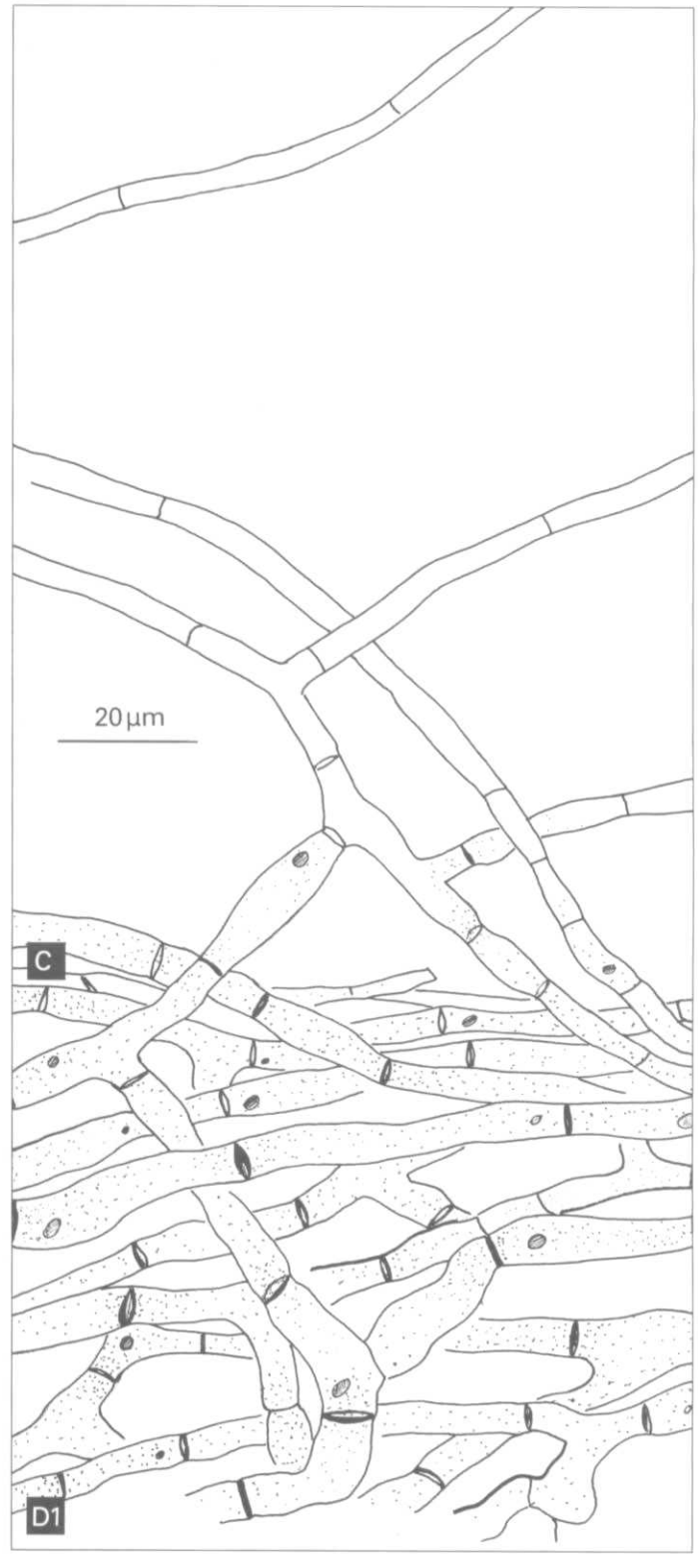
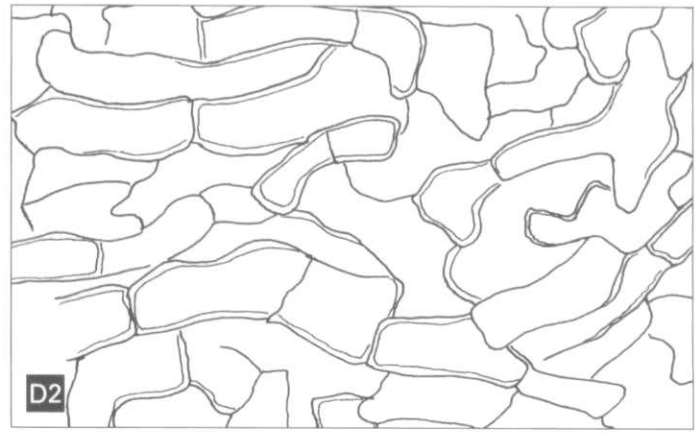

20 µm
C
D1


D2

Type: ITE.1

Identification:

Macroscopic appearance

Mycorrhizas are fairly straight with a frequent, sometimes pinnate, branching pattern. The main axis is <9 mm in length and <0.5 mm in diameter.

Occasionally, hyphae can be found extending far from the mantle surface, which appears smooth and shiny.

Mycorrhizas are white when young, changing to pale buff (52) with age.

Microscopic appearance

A. *Strands:* not observed.
B. *Sclerotia:* not observed.
C. *Mantle edge:* loosely formed.
Emanating hyphae: those closest to the mantle are 3–6 µm in diameter, distinctly septate and frequently branched. These hyphae may also be inflated, narrowing at the septa. Hyphae further away from the mantle become narrower (3–4 µm in diameter) and less frequently branched.
Specialised elements: not observed.
D. *Mantle:* 10–25 µm in depth.
   D1. *Surface:* a net prosenchyma of hyphae, 3–8 µm in diameter, frequently branched with septa staining strongly in lactophenol cotton blue or toluidine blue. These hyphae possess characteristically granular cytoplasm and large, globose, stained inclusions.
   D2. *Inner:* hyphae are broader (<15 µm in diameter) and shorter-celled (<20 µm in length) with thickened cell walls, forming a net synenchyma.

Distinguishing features

This mycorrhiza is distinguished by characteristics of hyphae forming the mantle surface. These hyphae closely resemble those associated with mycorrhizas described within the Humariaceae in this booklet. However, the better-developed mantle formed by this mycorrhiza suggests it may not be too closely related.

Ecology

Mycorrhizas have frequently been observed on *Picea sitchensis* (Bong.) Carr seedlings up to 5 years old growing in forest brown earth soils in the glasshouse.

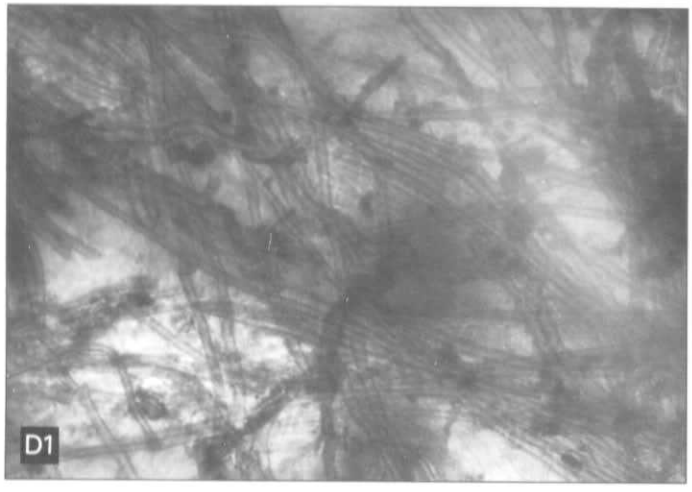
Associated trees:
Betula pendula
Picea sitchensis

Class:
Order:
Family:

4

Type: ITE.2


C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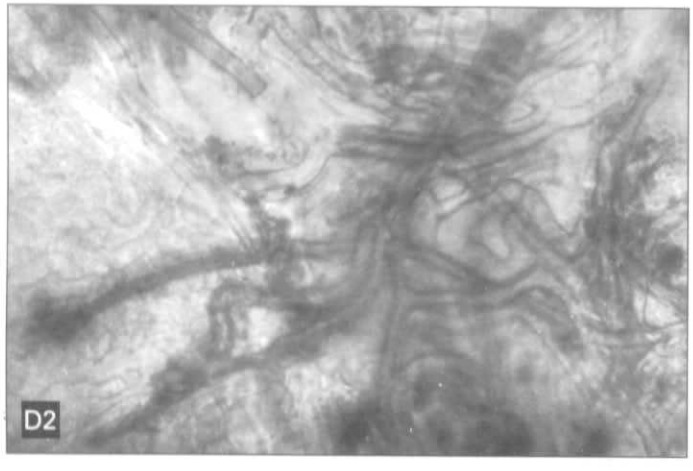
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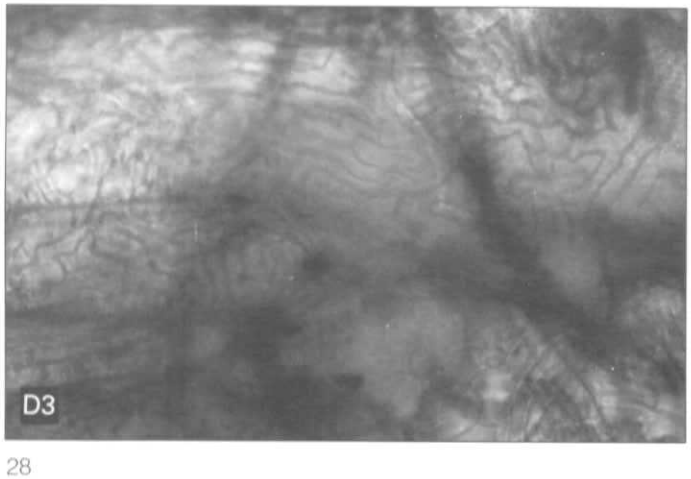
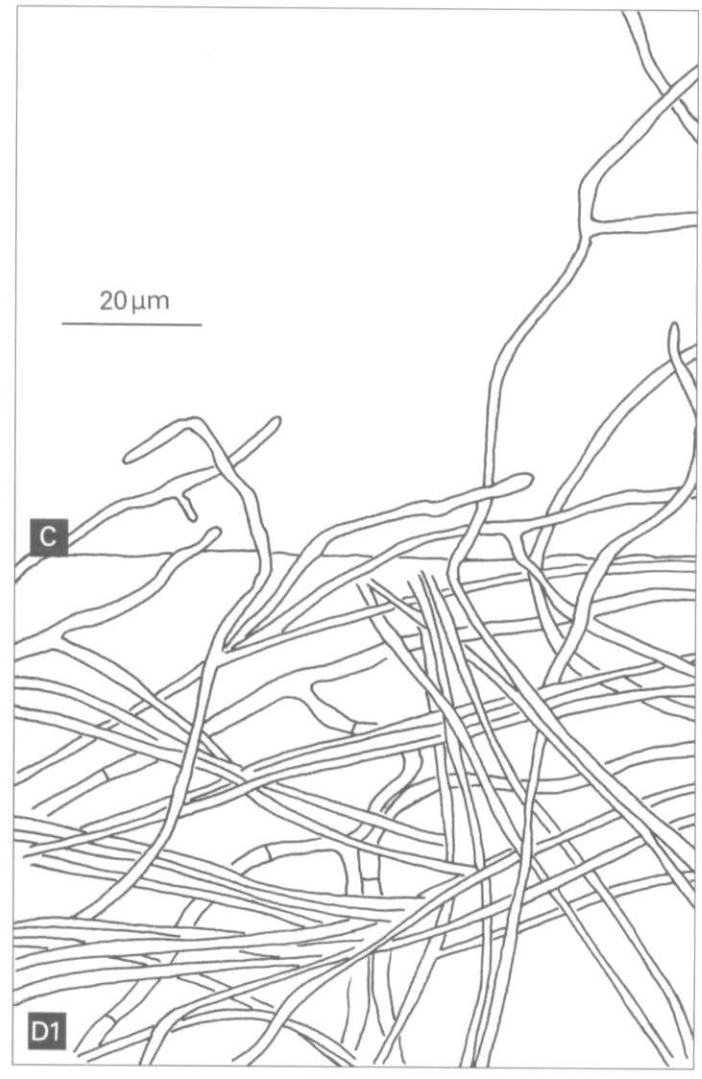
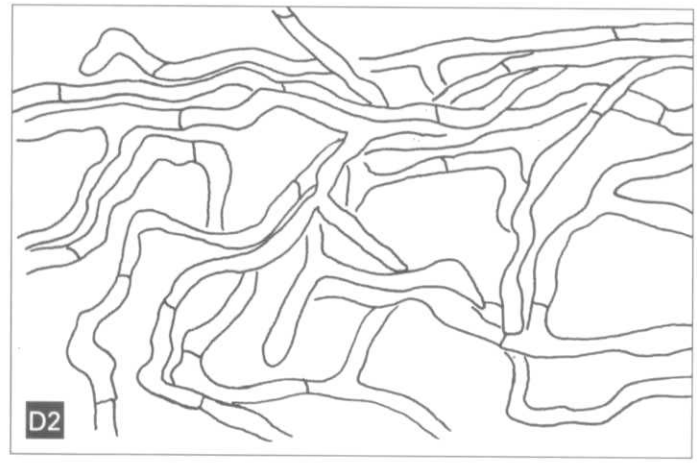

D3


20 µm
C
D1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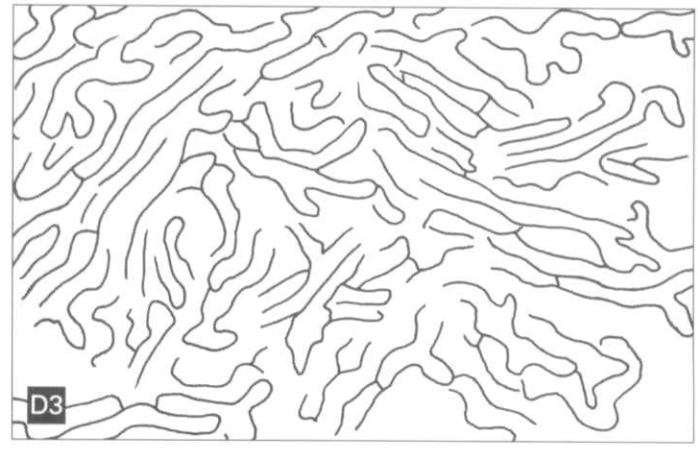

D3

Type: ITE.2

Identification:

Macroscopic appearance

Mycorrhizas are fairly long and straight with a frequent, sometimes pinnate, branching pattern. The main axis is <12 mm in length and <0.5 mm in diameter.

The mantle surface is smooth and shiny. Loose hyphae are frequently found and are just visible under the dissecting microscope.

Mycorrhizas are fulvous (12) when young, changing to snuff-brown (17) with age.

Microscopic appearance

A. *Strands:* not observed.
B. *Sclerotia:* not observed.
C. *Mantle edge:* loosely formed.
Emanating hyphae: narrow (1–2 µm in diameter), septate and frequently branched.
Clamp-connections absent.
Specialised elements: not observed.
D. *Mantle:* 5–10 µm in depth.
   D1. *Surface:* a felt prosenchyma of hyphae, like those emanating from the mantle, loosely arranged in interweaving parallel bands.
   D2. *Intermediate:* hyphae are broader (2–5 µm in diameter) and shorter-celled (<25 µm in length) than the surface hyphae. They are also more frequently branched and tortuous, interlocking to form a net prosenchyma.
   D3: *Inner:* hyphae are 2–4 µm in diameter and 2–10 µm in length, tortuous, and closely interlocking to form a net synenchyma.

Distinguishing features

The grey-brown colour and slender form of these mycorrhizas can be a useful distinguishing feature. However, they are best characterised by the presence and arrangement of the narrow hyphae, lacking clamp-connections which form the mantle surface. At present, we have no clues as to its identity.

Ecology

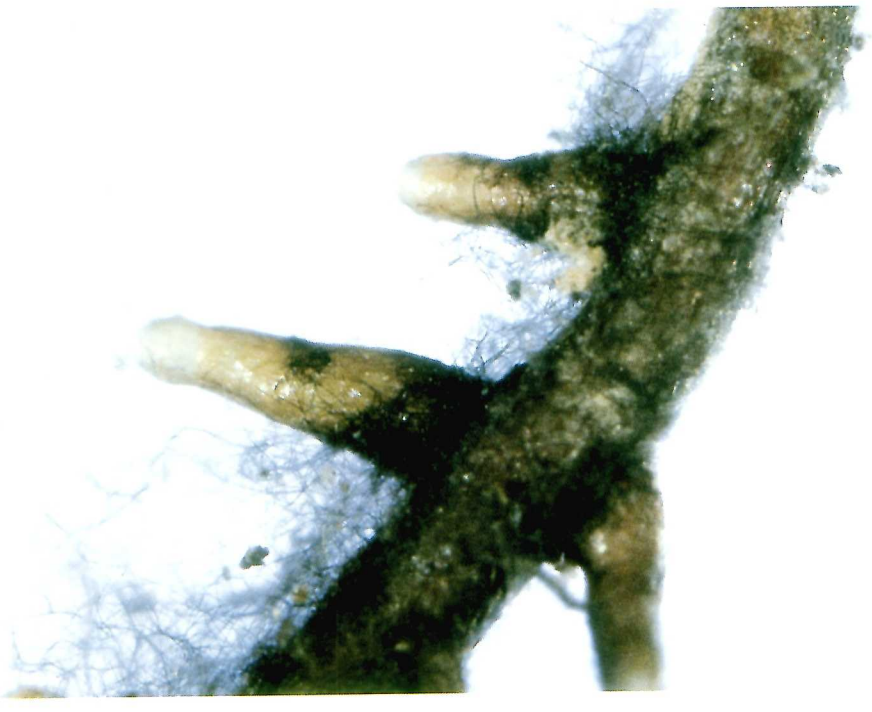
Mycorrhizas have been recorded on nursery seedlings, but have been found more frequently on *Betula* and *Picea* spp. seedlings 2–10 years old, growing in brown earth sites near Edinburgh.

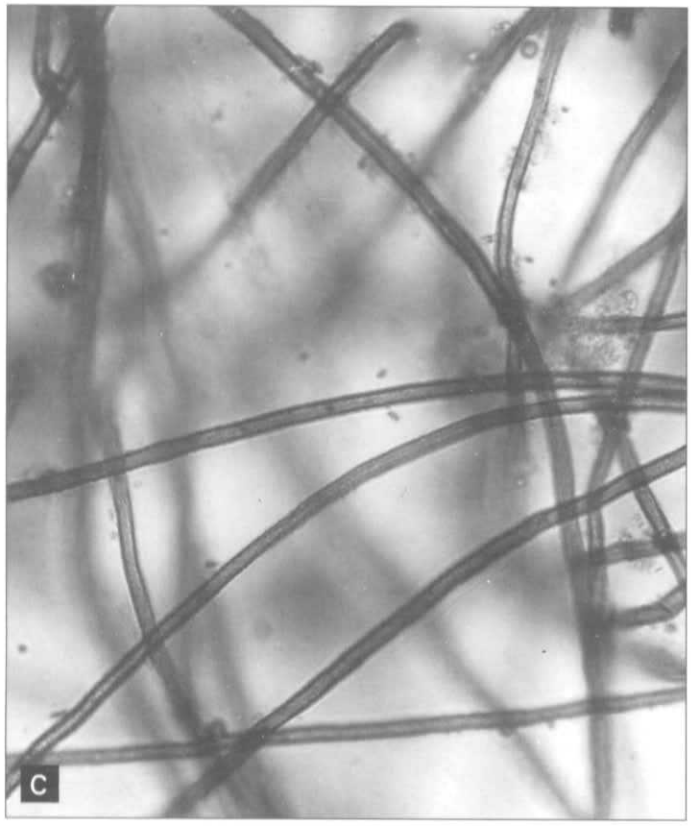
Associated trees:
Picea sitchensis
Pseudotsuga menziesii

Class: **DEUTEROMYCOTINA**
Order: **AGONOMYCETALES**
Family:

5

Type: ITE.3

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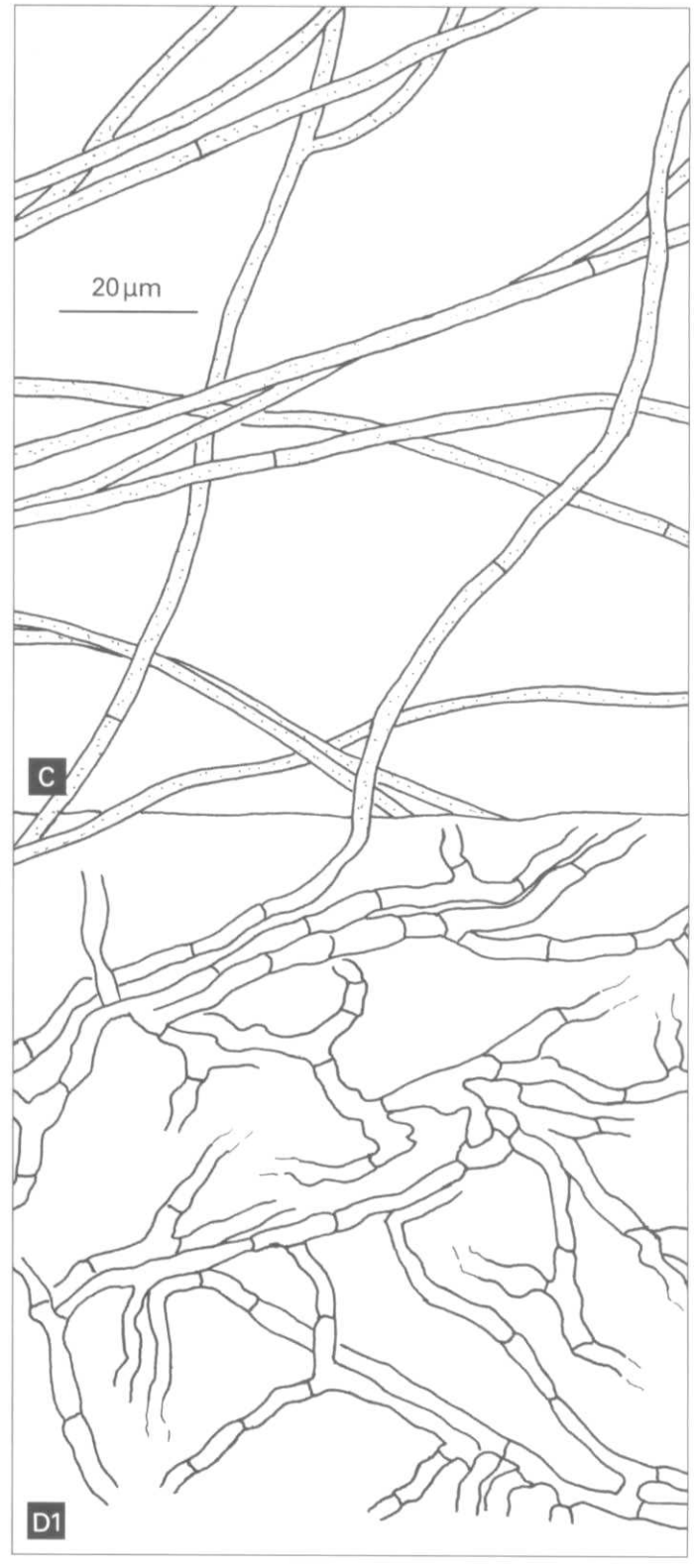
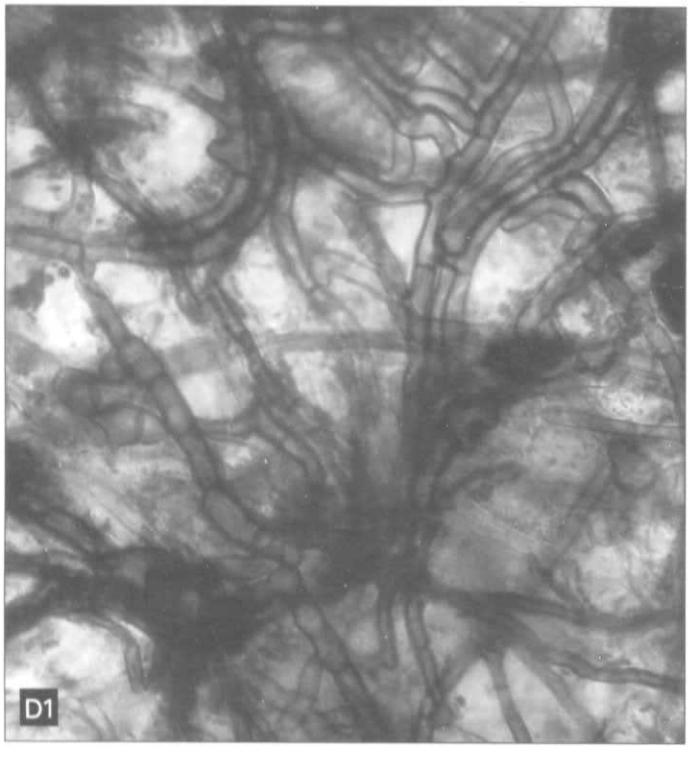

20 µm
C
D1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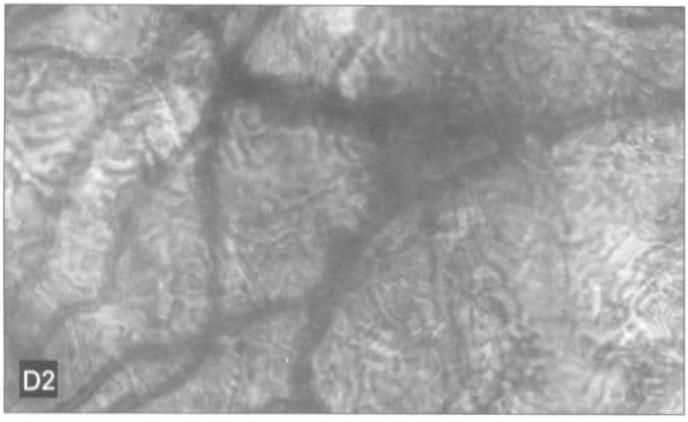
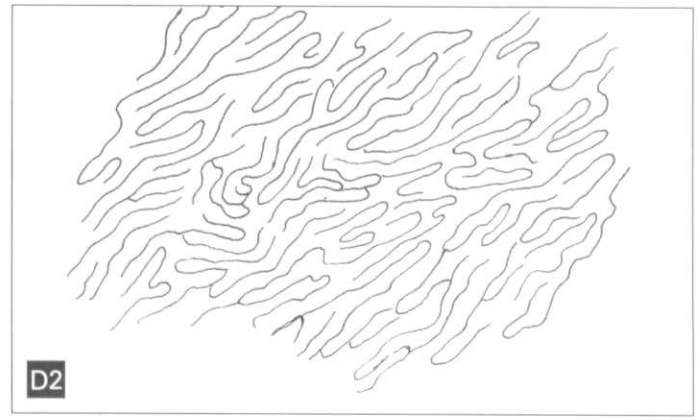

D2


D2

Type: ITE.3

Identification:
Literature descriptions

Macroscopic appearance

Mycorrhizas are short and very infrequently branched. The main axis is <5 mm in length and <0.5 mm in diameter.

Abundant black hyphae loosely surround the base of the mycorrhiza.

Mycorrhizas are brown at the tip where the mantle is absent, blackening towards the base as the mantle forms.

Microscopic appearance

A. *Strands:* not observed, although occasionally 2–3 hyphae may be seen very loosely connected.
B. *Sclerotia:* not observed.
C. *Mantle edge:* loosely formed.

Emanating hyphae: narrow (1.5–2.5 µm in diameter), septate, straight, infrequently branched and usually finely verrucose.
Specialised elements: not observed.

D. *Mantle:* 5 µm in depth, where present.
   D1. *Surface:* a net prosenchyma of black, septate hyphae frequently branched and banded together, becoming colourless with depth. The hyphal cells are often inflated, narrowing at the septa, and are 2–5 µm in diameter. The mantle is often incomplete and rarely more than 2–3 cells in depth.
   D2. *Hartig net:* a compact, fine, labyrinthine structure of hyphae 1.0–2.5 µm in diameter. It is always visible towards the root tip, where the mantle is absent.

Distinguishing features

This mycorrhiza is one of a group of brown-black mycorrhizas, distinguished by its hyphal characteristics and a weakly developed mantle. From its occurrence and characteristics, it would appear that this fungus is one of several species of Agonomycetales included under the name of *Mycelium radicis atrovirens* Melin. Further definition has not been possible as sporulation structures have not yet been observed associated with these mycorrhizas.

Ecology

This mycorrhiza is common on nursery seedlings, persisting on the roots after outplanting. It is particularly dominant when large numbers of moribund roots are present, suggesting it may be of a slightly pathogenic nature.

References

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WANG, C.J.K. & WILCOX, H.E. 1985. New species of ectendomycorrhizal and pseudomycorrhizal fungi: *Phialophora finlandia, Chlorodium paucisporum,* and *Phialocephala fortinii. Mycologia,* **77,** 951–958.

WILCOX, H.E., GANMORE-NEUMANN, R. & WANG, C.J.K. 1974. Characteristics of two fungi producing ectendomycorrhizae in *Pinus resinosa. Can. J. Bot.,* **52,** 2279–2282.

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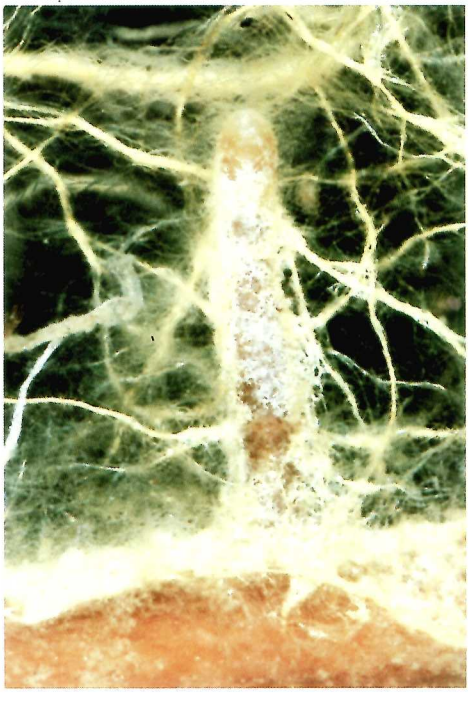
Associated trees:
Picea sitchensis
Pseudotsuga menziesii

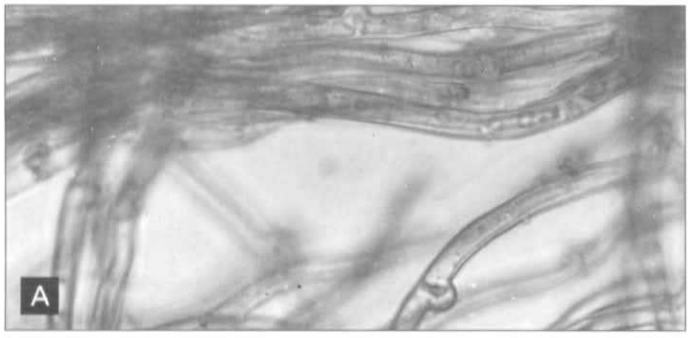
Class: **BASIDIOMYCOTINA**
Order: **APHYLLOPHORALES**
Family: **CORTICIACEAE**

6

Amphinema byssoides

(Pers.: Fr.) Erikss.

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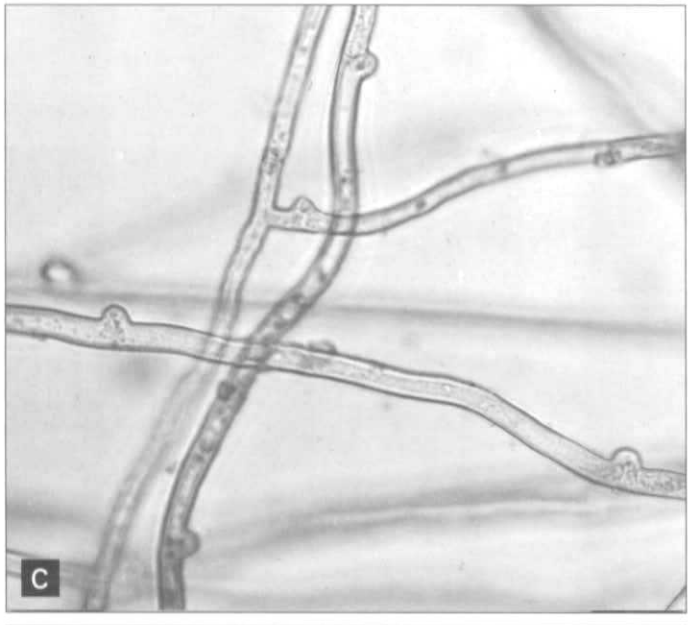
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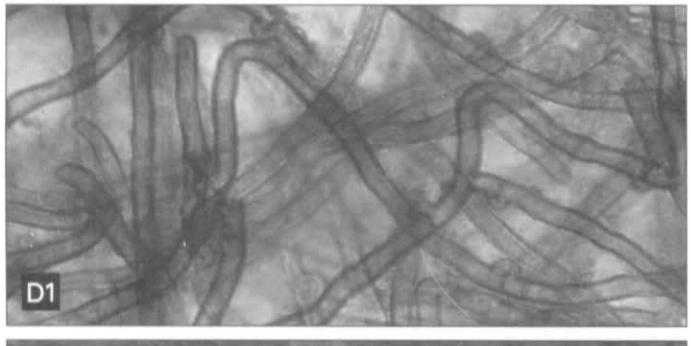
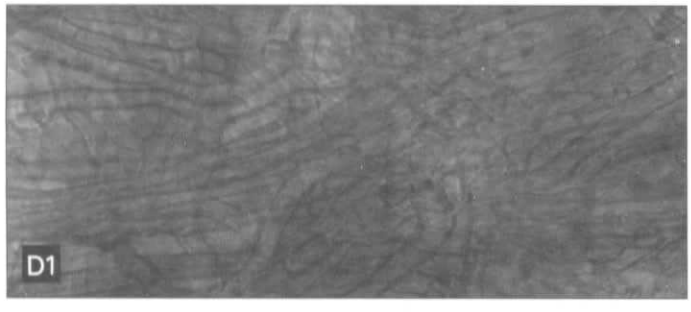

D1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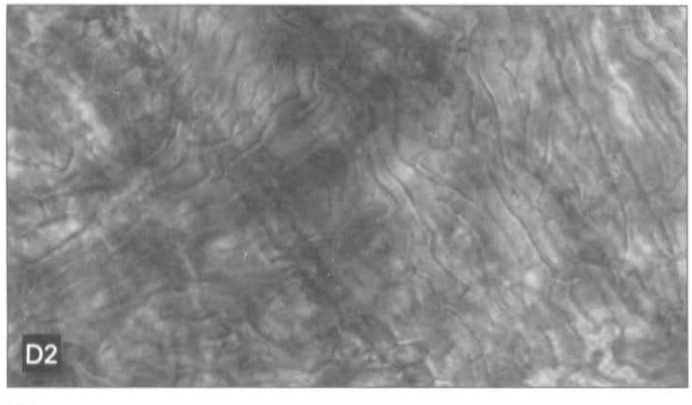
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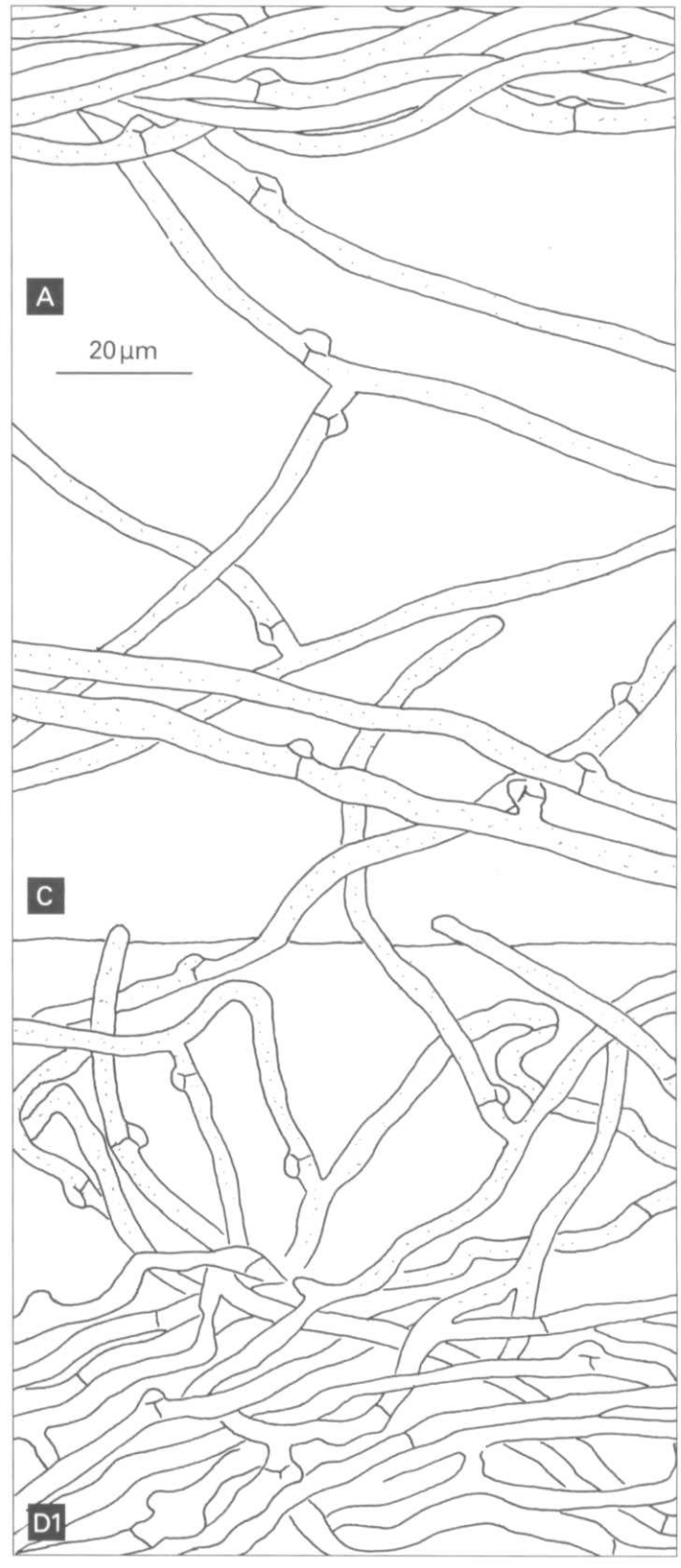
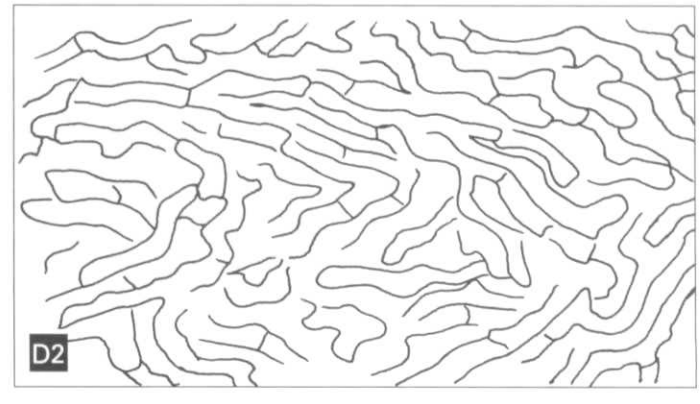

A
20 µm
C
D1


D2

Amphinema byssoides

(Pers.: Fr.) Erikss.

Identification:
Fruitbody links, literature descriptions

Macroscopic appearance

Mycorrhizas are fairly straight, slender and infrequently branched. The main axis is <8 mm in length and <0.4 mm in diameter.

Dense, abundant wefts of hyphae and simple strands (<0.2 mm diameter) are found, emanating from the mantle surface.

Young mycorrhizas, hyphae and strands are silver-straw (50) to silver-lemon chrome (53), older mycorrhizas becoming dark rusty-brown.

Microscopic appearance

A. *Strands:* undifferentiated, very loosely formed, composed of hyphae (3–5 µm in diameter) which are finely verrucose with abundant, often loosely formed clamp-connections.
B. *Sclerotia:* not observed.
C. *Mantle edge:* loosely formed.

Emanating hyphae: like those associated with the strands, 2–4 µm in diameter, finely verrucose with abundant clamp-connections.
Specialised elements: not observed.
D. *Mantle:* 15–30 µm in depth.
   D1. *Surface:* hyphae like those emanating from the surface, forming a felt prosenchyma. These hyphae become compacted with age, forming a net synenchyma.
   D2. *Inner:* a net synenchyma of interwoven, hyphal cells, 5–40 µm in length and 1–4 µm in diameter.

Distinguishing features

This mycorrhiza is most easily distinguished on macroscopic features of yellow-tinted mycorrhizas, wefts of hyphae, and loosely formed strands.

The mycorrhizas are microscopically similar to several species of *Hebeloma* mycorrhizas, with a thin, poorly developed mantle and hyphae with abundant clamp-connections. However, differentiation can be made by the finer encrustation of *A. byssoides* hyphae and also by the more uniform, hemispherical protrusion of *Hebeloma* spp. clamp-connections.

Mycorrhizas of the related fungus *Piloderma croceum* Erikss. & Hjorst. have been examined and found to be very similar. However, they can be distinguished macroscopically by the deeper yellow coloration of the mycorrhiza and associated mycelium, and microscopically by hyphae which do not possess clamp-connections.

Ecology

Mycorrhizas have frequently been observed on *Picea sitchensis* (Bong.) Carr seedlings growing in nursery soils and glasshouse potting composts. They are also recorded in association with *Pseudotsuga menziesii* (Mirb.) Franco, *Picea glauca* (Moench.) Voss. and *Pinus strobus* L.

Fruitbody observations support the view that this fungus is commonly associated with a wide range of coniferous hosts.

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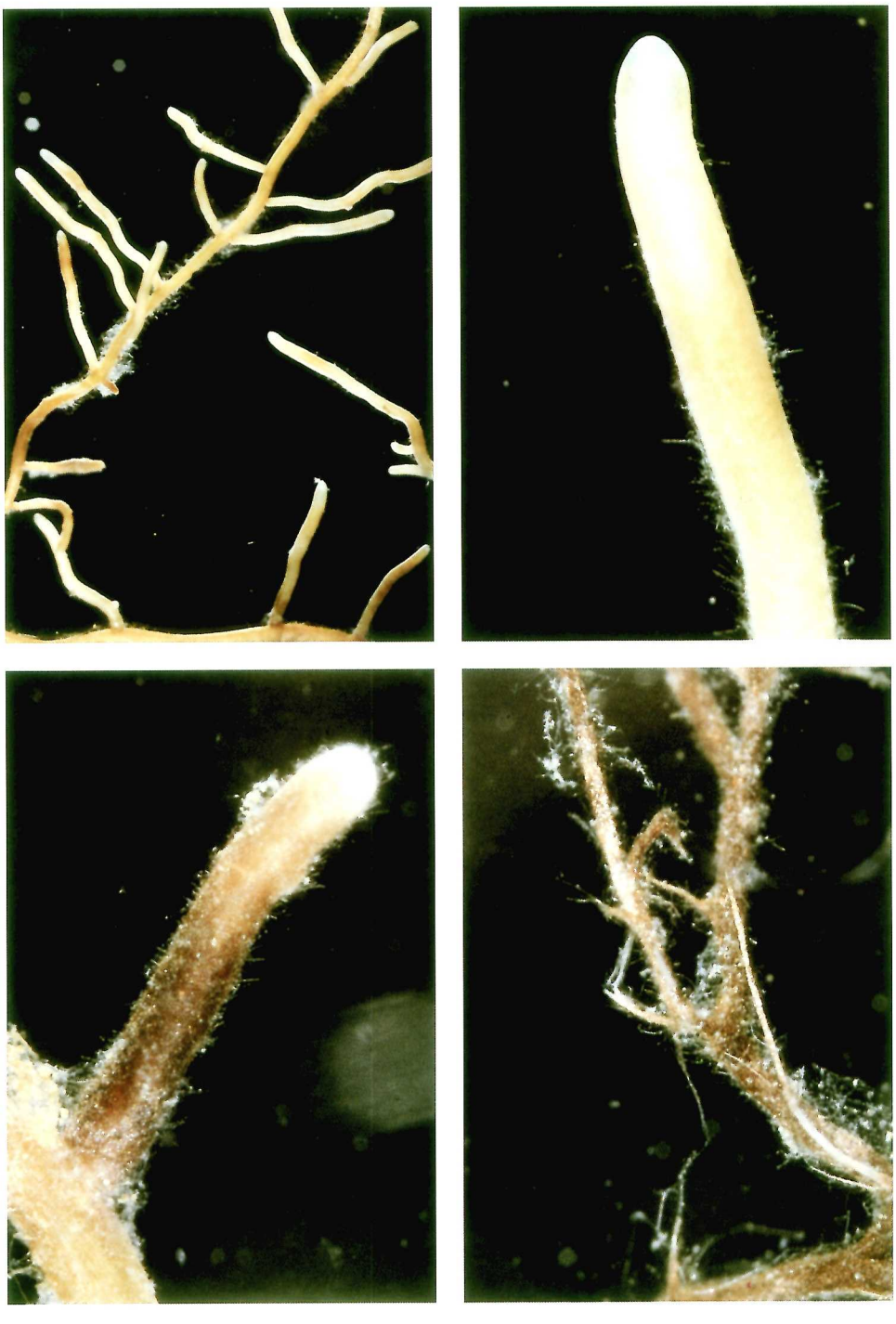
Associated trees:
Betula pendula
Betula pubescens
Picea sitchensis
Pinus contorta
Pinus sylvestris

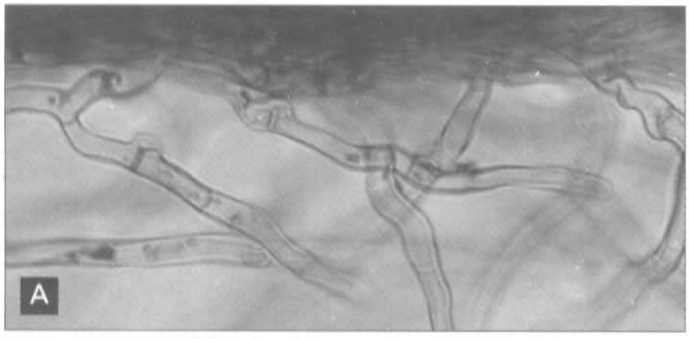
Class: **BASIDIOMYCOTINA**
Order: **APHYLLOPHORALES**
Family: **THELEPHORACEAE**

7

Thelephora terrestris

(Ehrh.) Fr.

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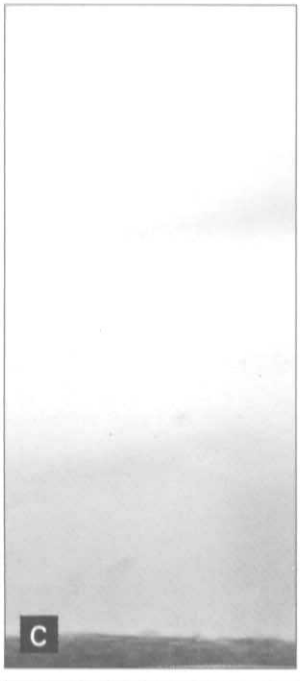
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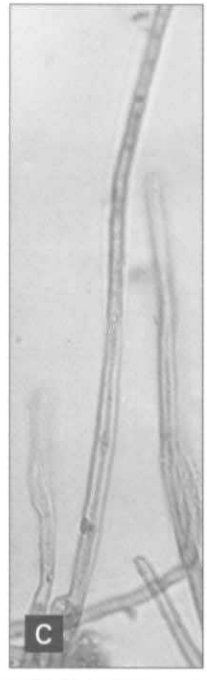
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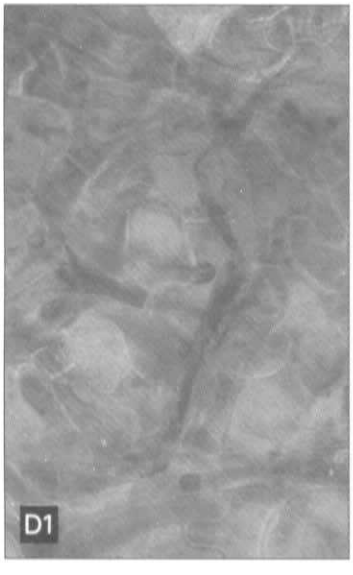
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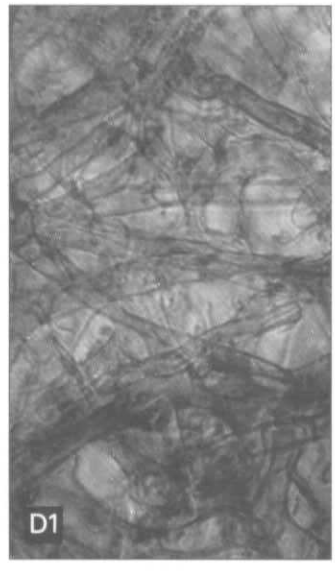
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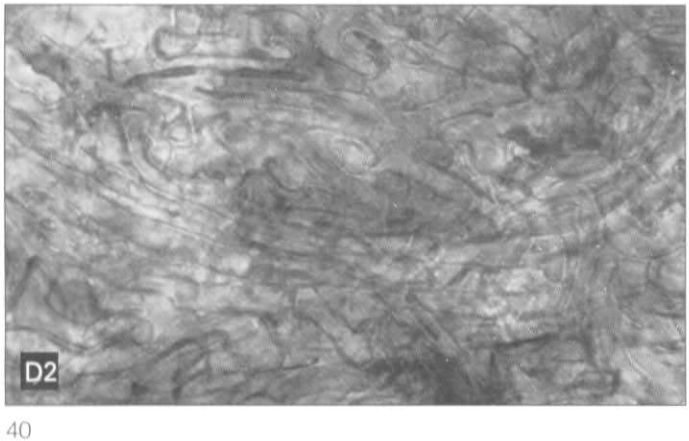
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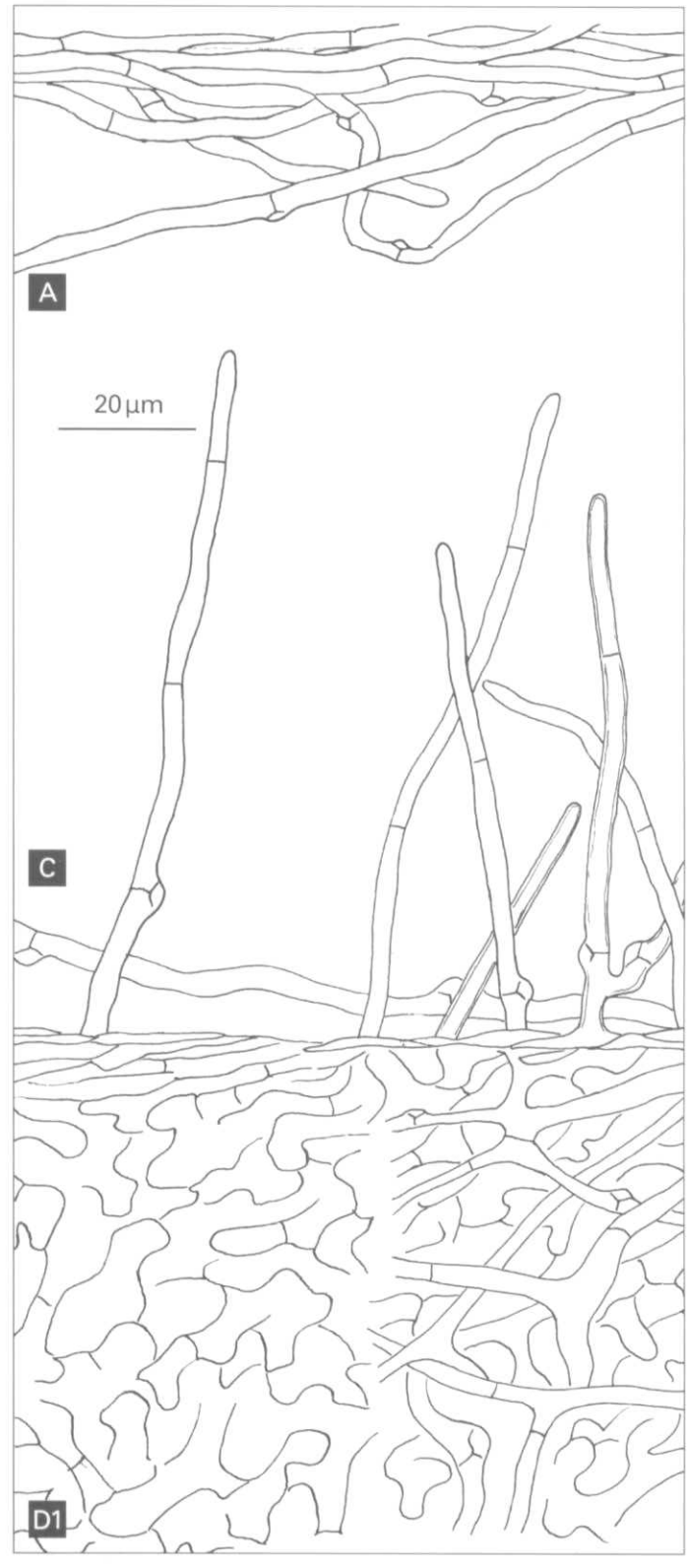
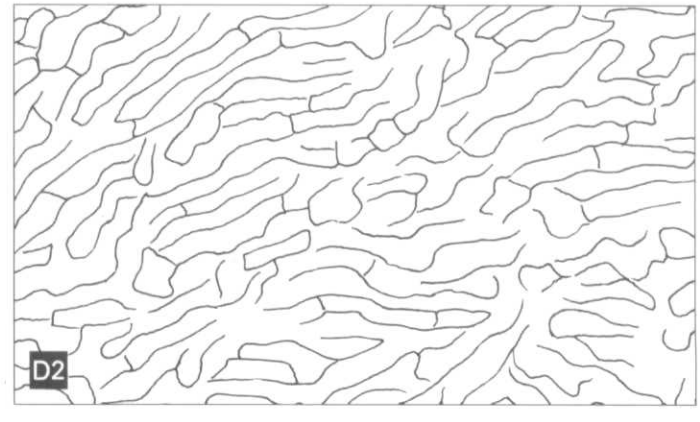

A
20 µm
C
D1


D2

Thelephora terrestris

(Ehrh.) Fr.

Identification:
Synthesis, fruitbody links, literature descriptions

Macroscopic appearance

Mycorrhizas are fairly long and sinuous, with frequent, irregularly spaced, short branches. The main axis is <10 mm in length and <0.5 mm in diameter.

Fringes of short, straight, differentiated hyphae, <0.2 mm in length, are often found on the mantle surface and can just be discerned under the dissecting microscope. Thin strands (<0.1 mm in diameter) are also present.

Mycorrhizas of *T. terrestris* exhibit two distinctive forms. One, a pale form, is white when young, turning fawn to darker shades of orange or grey-brown with age. Differentiated hyphae, although always present, are thinly spread on the mantle surface, and strands, concolorous with the mantle, are infrequently found. Mycorrhizas of the second form are silver-dark brick (20) when young, turning to purplish chestnut (21) with age. This form is characterised by a dense fringe of differentiated hyphae covering the entire mantle surface and abundant strand formation, again concolorous with the mantle. Although populations of mycorrhizas may consist entirely of either of these two forms, it is just as common for both to occur within the same root system, and even for both to occur in patches along a single mycorrhiza.

Microscopic appearance

A. *Strands:* undifferentiated and loosely organised, composed of hyphae 2.5–3.5 µm in diameter with frequent clamp-connections. In older strands, these hyphae become yellow in colour with slightly thickened cell walls.

Associated hyphae: 2.5–4.0 µm in diameter with frequent clamp-connections.

B. *Sclerotia:* not observed.

C. *Mantle edge:* smooth and compact when not obscured by a dense covering of differentiated hyphae.

Specialised elements: differentiated hyphae 1.5–3.0 µm in diameter, straight or slightly sinuous and <150 µm in length. They are septate, but only the basal septum possesses a clamp-connection. These hyphae may be branched, with each branch possessing the basal clamp-connection. Differentiated hyphae associated with the pale form are hyaline, while those associated with the dark form are yellow with slightly thickened walls.

Emanating hyphae: found running horizontal to the mantle surface, tortuous, 2.5–4.0 µm in diameter with frequent clamp-connections.

D. *Mantle:* 10–30 µm in depth.

D1. *Surface:* an interlocking irregular synenchyma of cellular and hyphal elements <20 µm in length and <10 µm in diameter. The surface may be difficult to view in the dark form when overlain by a dense covering of differentiated hyphae flattened as a result of slide preparation.

D2. *Inner:* a net synenchyma of cells <40 µm in length and <10 µm in diameter.

Distinguishing features

This mycorrhiza is best distinguished on characteristics of differentiated hyphae and compact mantle surface. They compare very closely with those described by Danielson, Zak and Parkinson (1984) for mycorrhizas of the closely related *Tomentella* genus. In very young mycorrhizas, which possess a mantle surface not yet compacted, care should be taken not to confuse with those of *Laccaria proxima* (Boud.) Pat.

The two forms of *T. terrestris* appear quite different when viewed under the dissecting microscope, but examination of a root squash shows that features of differentiated hyphae and mantle structure remain constant in both forms. Interestingly, isolates cultured from *T. terrestris* fruitbodies exhibit similar 'pale' and 'dark' forms of growth in liquid and solid culture.

Ecology

Mycorrhizas are particularly abundant on containerised seedlings of glasshouse and nursery, and have also been commonly found on coal waste sites.

Fruitbody observations support the view that this is a very common, non-specific, 'early stage' fungus growing in a wide range of habitats.

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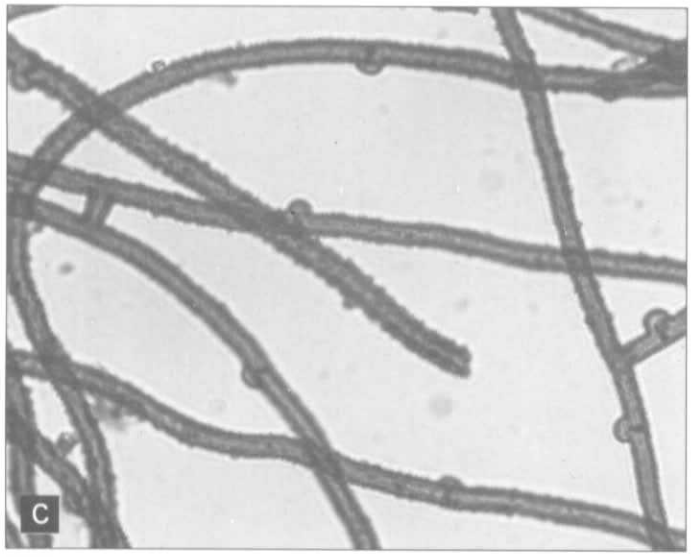
Associated trees:
Betula pendula
Picea sitchensis
Pinus contorta

Class: **BASIDIOMYCOTINA**
Order: **AGARICALES**
Family: **CORTINARIACEAE**

8

Hebeloma mesophaeum

(Pers.) Quél.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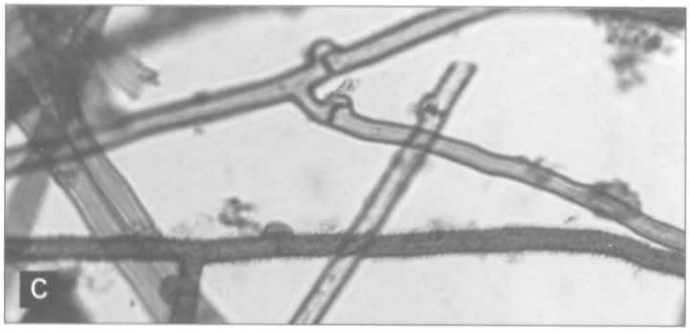
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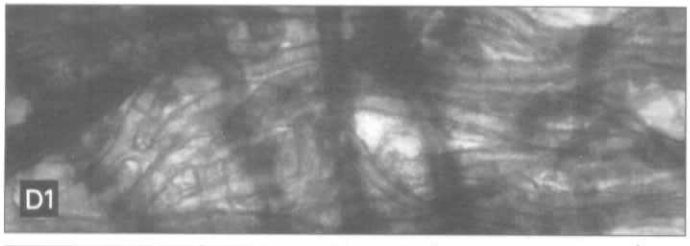
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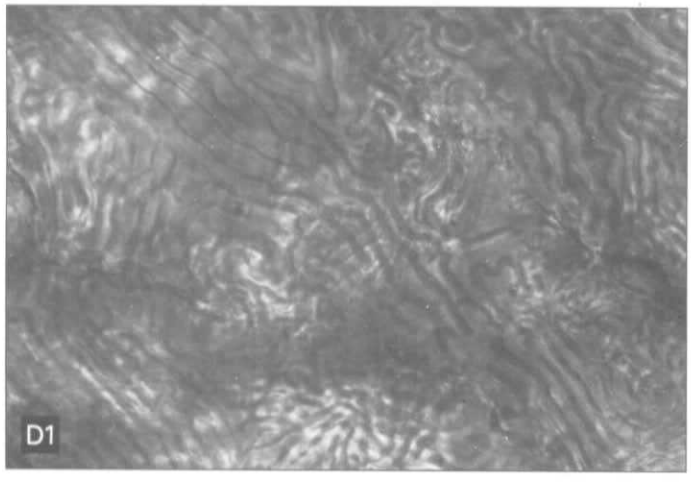
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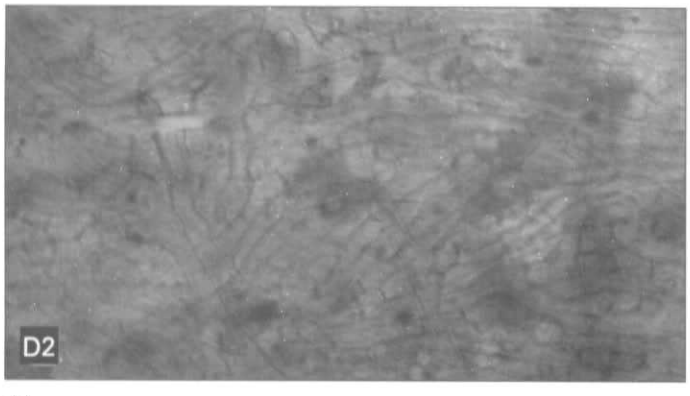
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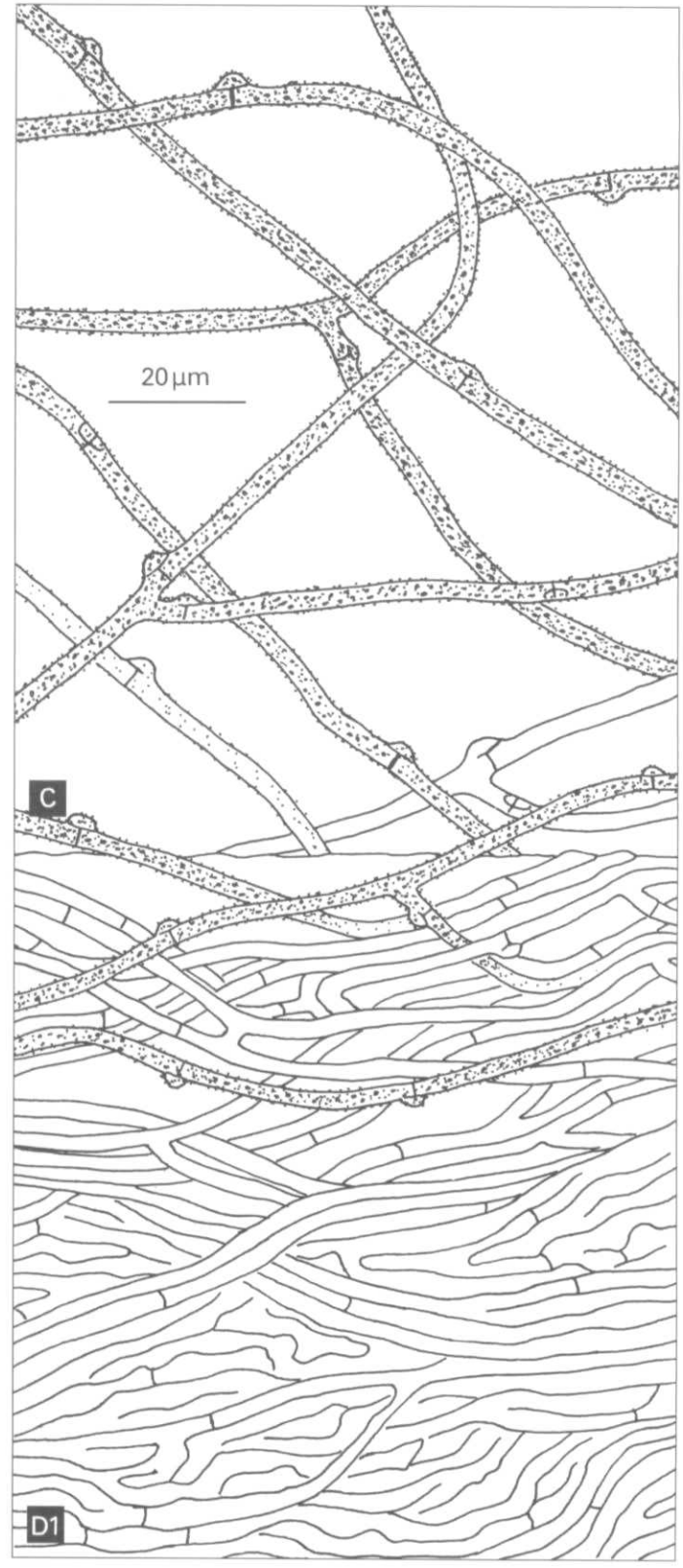
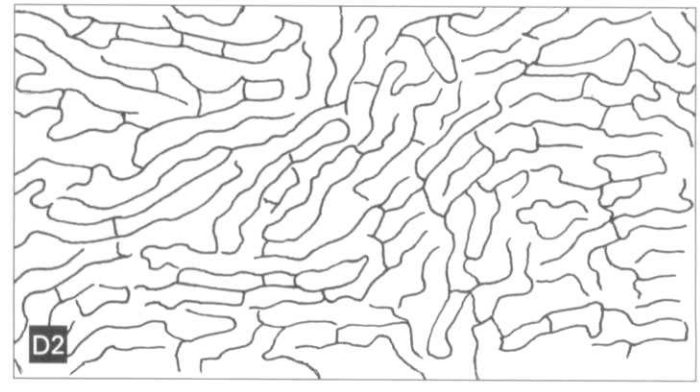

20 µm
C
D1


D2

Hebeloma mesophaeum
(Pers.) Quél.

Identification:
Synthesis, fruitbody links, literature descriptions

Macroscopic appearance

Mycorrhizas are fairly long and slender, and infrequently branched. The main axis is <10 mm in length and <0.4 mm in diameter.

Dense, abundant hyphae are invariably found surrounding the mantle.

Mycorrhizas are silver-white when young, rapidly changing to rusty-tawny (14) with age.

Microscopic appearance

A. *Strands:* not observed.
B. *Sclerotia:* not observed.
C. *Mantle edge:* loosely formed.
Emanating hyphae: 3–4 µm in diameter with uniform, tightly formed, hemispherical clamp-connections at most septa. The hyphae are frequently branched, often at an angle of 90° and those not closely associated with the mantle are usually distinctly verrucose. These hyphal encrustations stain dark blue (73) in toluidine blue.
Specialised elements: not observed.
D. *Mantle:* 5–20 µm in depth.
   D1. *Surface:* when young, a felt prosenchyma of hyphae similar to those emanating from the surface. However, it matures rapidly to a net synenchyma composed of distinctive parallel bands of diverging hyphae (2–4 µm in diameter), which are not verrucose.
   D2. *Inner:* a net synenchyma of shortened, tortuous hyphal cells <20 µm in length and <5 µm in diameter.

Distinguishing features

This mycorrhiza is distinguished from the other *Hebeloma* species described in this booklet by the more distinctive 'banding' of the mantle surface hyphae and the verrucose nature of the emanating hyphae.

Care should also be taken to distinguish these mycorrhizas from those of *Amphinema byssoides* (Pers.: Fr.) Erikss., which are macroscopically distinguished by their yellow colour and loose strand-forming habit. Microscopically, differentiation can be made by the finer encrustation of *A. byssoides* hyphae and the more uniform, hemispherical protrusion of *Hebeloma* spp. clamp-connections.

Ecology

Like all species of *Hebeloma* we have observed, these mycorrhizas are frequently encountered on a wide range of young tree species, and may be considered 'early stage' fungi. They are particularly common on trees growing in reasonably fertile brown earth soils, tree nurseries and glasshouse potting composts. Under such conditions, *Hebeloma* mycorrhizas will dominate root systems and readily produce fruitbodies.

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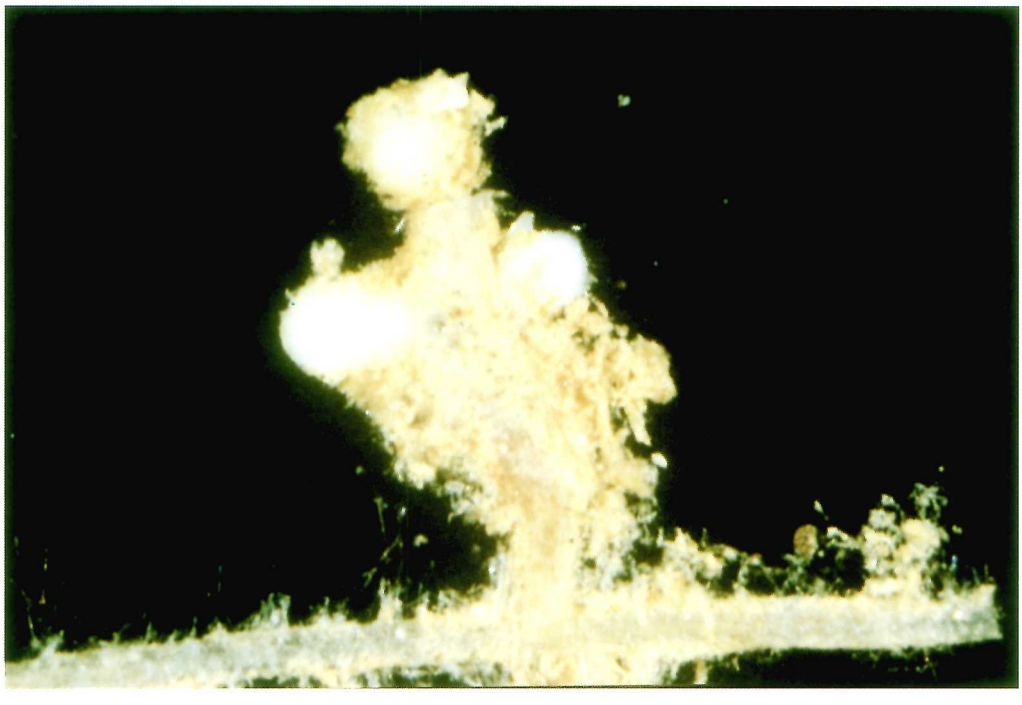
Associated trees:
Betula pendula
Picea sitchensis

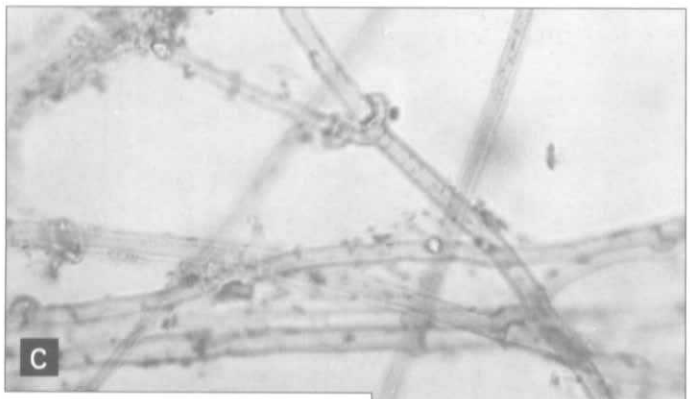
Class: **BASIDIOMYCOTINA**
Order: **AGARICALES**
Family: **CORTINARIACEAE**

9

Hebeloma sacchariolens

Quél.

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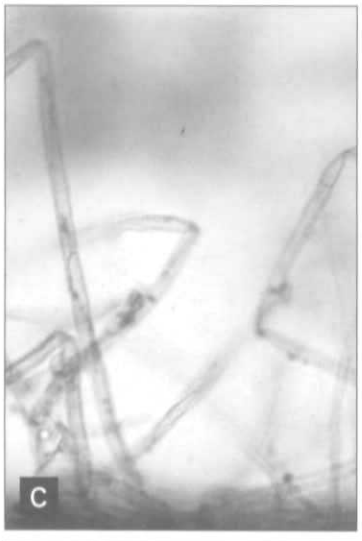
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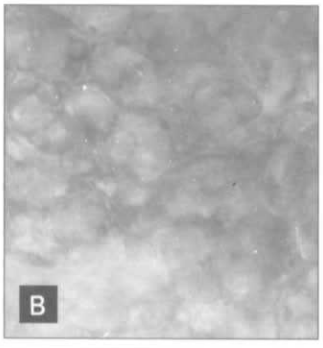
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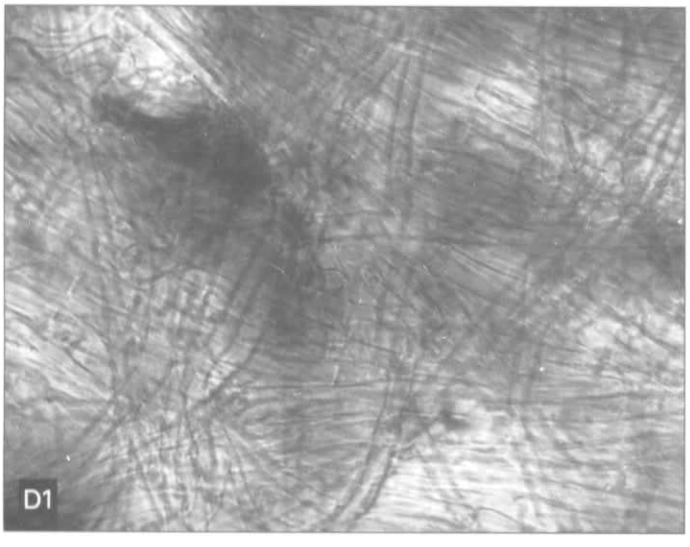
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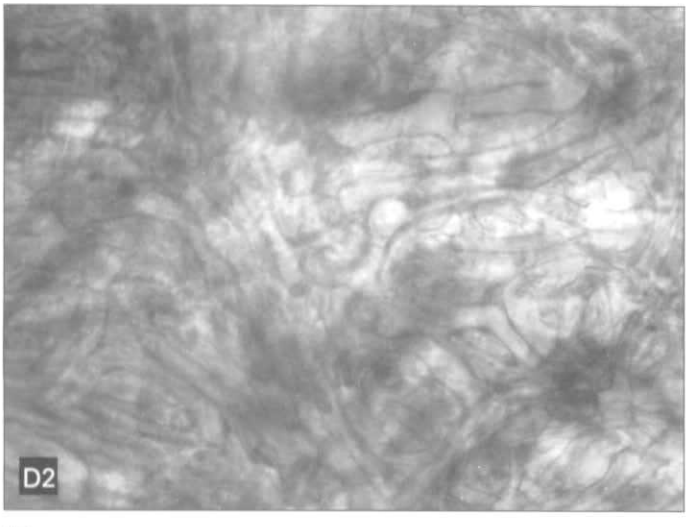
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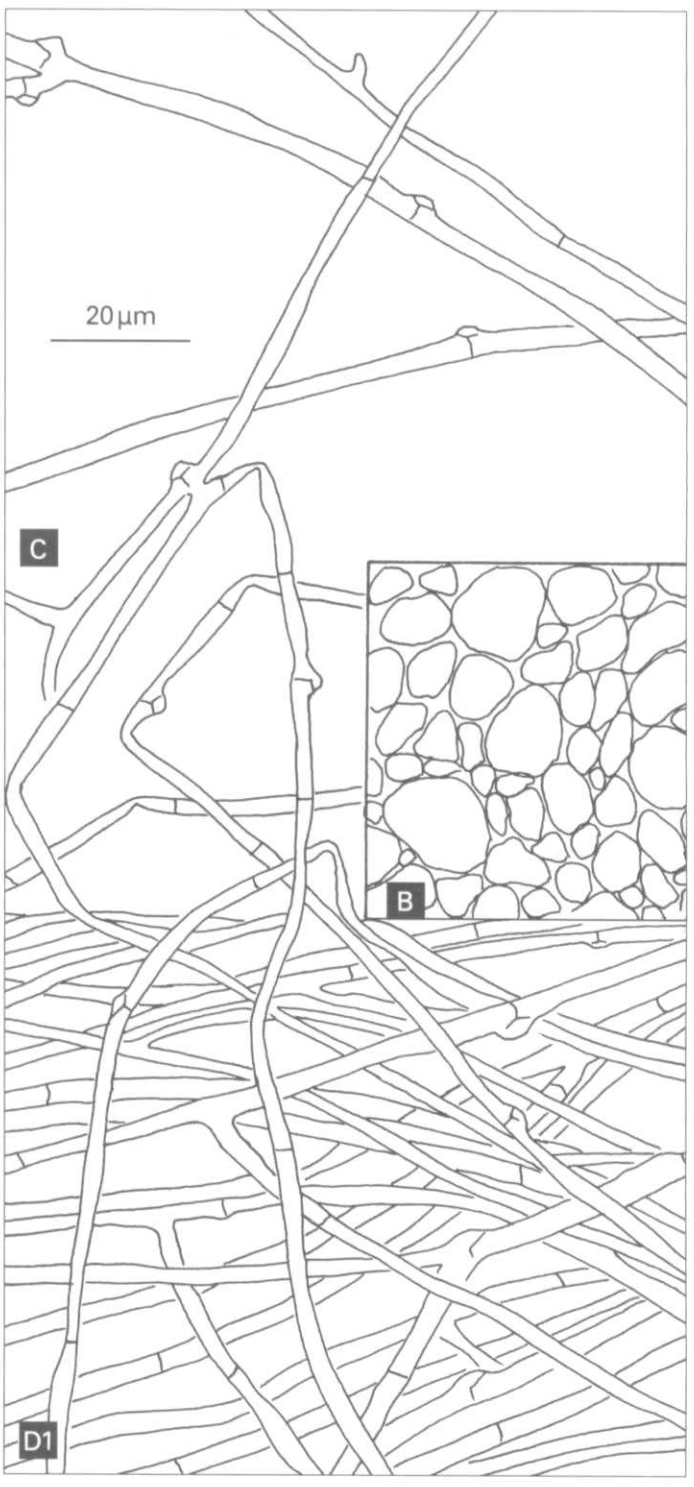
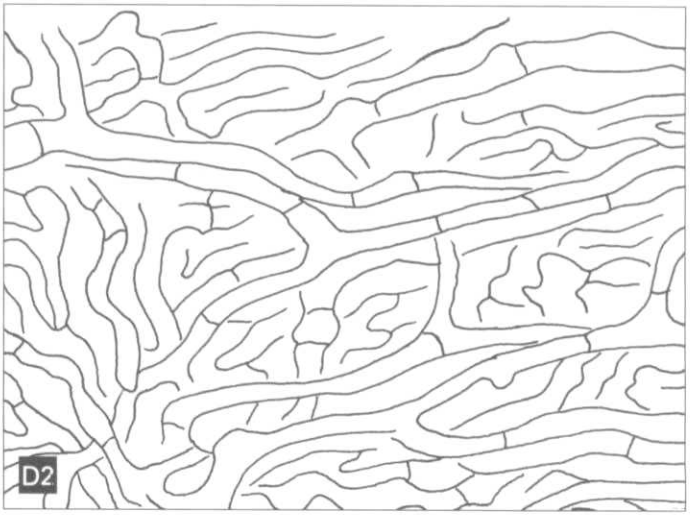

20 µm
C
B
D1


D2

Hebeloma sacchariolens

Quél.

Identification:
Synthesis, fruitbody links

Macroscopic appearance

Mycorrhizas are short, slender and very infrequently branched. The main axis is <5 mm in length and <0.3 mm in diameter.

Conspicuous white sclerotia are always found adhering to the mantle surface. These sclerotia are 0.2–0.4 mm in diameter, globose, subglobose or flattened in appearance. Abundant hyphae surround the mantle, but usually do not extend more than 0.2 mm from the surface. Soil particles are particularly difficult to remove from these hyphae, making them difficult to observe either macroscopically or microscopically.

Mycorrhizas are silver-white when young, changing to grey-brown with age.

Microscopic appearance

A. *Strands:* not observed.
B. *Sclerotia:* composed of a very thin outer layer of loose hyphae which, when squashed, reveal a compact layer of lipid-containing, subglobose or slightly angular cells, 3–10 µm in diameter.
C. *Mantle edge:* loosely formed. Emanating hyphae 2–4 µm in diameter, frequently branched, with uniform, tightly formed, hemispherical clamp-connections at most septa. These hyphae are rarely verrucose.

Specialised elements: not observed.

D. *Mantle:* 5–15 µm in depth.
   D1. *Surface:* a felt prosenchyma composed of hyphae like those emanating from the mantle surface. These hyphae become slowly compacted with age, and possess fewer clamp-connections.
   D2. *Inner:* a net synenchyma of cells, <80 µm in length and <7 µm in diameter.

Distinguishing features

Mycorrhizas of *H. sacchariolens* can be distinguished macroscopically from all other *Hebeloma* spp. we have encountered by the presence of large sclerotia firmly attached to the mantle surface, and by the lack of dense, abundant, emanating hyphae extending far from the mantle surface.

However, near-identical microscopic characteristics of hyphae and mantle have been observed on mycorrhizal populations tentatively linked with fruitbodies of *Hebeloma crustuliniforme* (Bull.: St Amans) Quél., *Hebeloma leucosarx* P.D. Orton, and *Hebeloma velutipes* Bruchet. Although we have not been able to differentiate these mycorrhizas, Zak (1973) and Voiry (1981) have described *H. crustuliniforme* mycorrhizas as possessing tiny white sclerotium-like bodies loosely attached to the extramatrical mycelium.

Mycorrhizas of *Hebeloma mesophaeum* (Pers.) Quel. are distinguished by the more distinctive 'banding' of the mantle surface hyphae and the verrucose nature of the emanating hyphae.

Ecology

Mycorrhizas have frequently been recorded on young *Betula* spp. of 2–10 years of age, growing in brown earth soils near Edinburgh. Persistence of these mycorrhizas following inoculation of tree seedlings in unsterile soils indicates that it is an 'early stage' fungus.

Although mycorrhizas have been recorded on *Picea sitchensis* (Bong.) Carr, fruitbody observations suggest that this fungus is most commonly associated with *Betula* and *Salix* spp.

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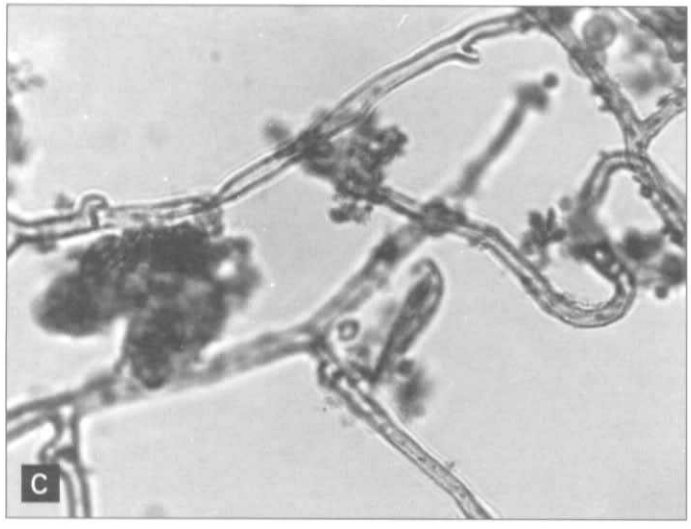
Associated trees:
Betula pendula
Betula pubescens
Picea sitchensis
Pinus contorta
Pinus sylvestris

Class: **BASIDIOMYCOTINA**
Order: **AGARICALES**
Family: **TRICHOLOMATACEAE**

10

Laccaria proxima

(Boud.) Pat.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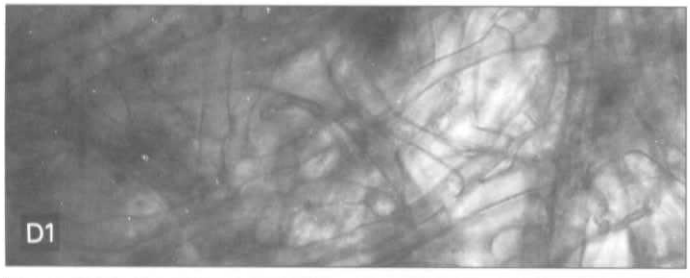
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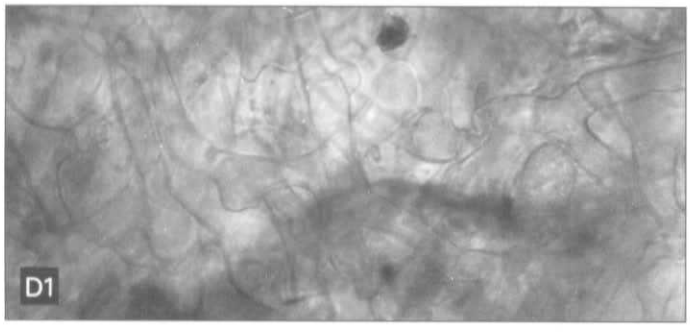
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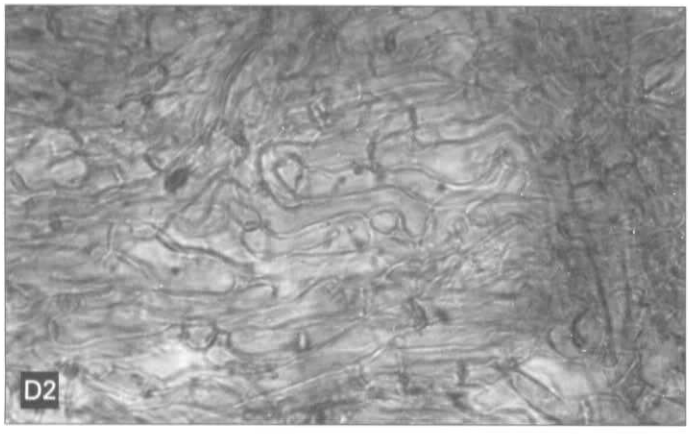
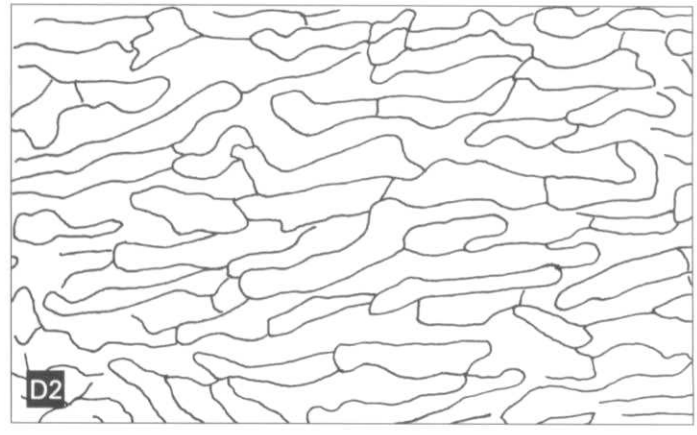

D2


20 µm
C
D1


D2

Laccaria proxima

(Boud.) Pat.

Identification:
Synthesis, fruitbody links, literature descriptions

Macroscopic appearance

Mycorrhizas are fairly long and sinuous, with frequent, irregularly spaced, short branches. The main axis is <8 mm in length and <0.6 mm in diameter.

Loose, straggly, hyphae can frequently be seen close to the mantle surface.

Mycorrhizas are usually white to buff when young, darkening with age to various shades of orange or grey-brown. All mycorrhizas retain an opaque milk-white lustre.

Microscopic appearance

A. *Strands:* not observed.
B. *Sclerotia:* not observed.
C. *Mantle edge:* loosely formed becoming compacted in older mycorrhizas. Emanating hyphae: tortuous, 2.5–4.0 µm in diameter, with abundant, large, irregularly formed clamp-connections and elbow-like protrusions. Hyphae found closest to the mantle surface tend to be of slightly larger diameter (3.5–5.0 µm). Specialised elements: not observed.
D. *Mantle:* 15–40 µm in depth.
   D1. *Surface:* a net prosenchyma composed of loosely interwoven hyphae (2.5–5.0 µm in diameter), with occasional clamp-connections. Hyphae in the young mantle stain strongly blue in lactophenol cotton blue, and tend to run parallel to each other along the axis of the mycorrhiza. Hyphae broaden (<8 µm in diameter) and interlock more closely with age to form a net synenchyma.
   D2. *Inner:* a net synenchyma of tortuous lobate cells, <20 µm in length and <6 µm in diameter.

Distinguishing features

This mycorrhiza is not easy to distinguish from other *Laccaria* spp., and even from very young mycorrhizas of *Thelephora terrestris* (Ehrh.) Fr. which have not developed a compact mantle surface. However, it is best distinguished by characteristics of hyphae and young mantle surface stained in lactophenol cotton blue. The mantle structure closely resembles our description of *Laccaria tortilis* (Bolt.) S.F. Gray and also that of Brand and Agerer (1986) for *Laccaria amethystea* (Bull.: Mérat) Murr.

Ecology

Mycorrhizas are particularly common on containerised glasshouse and nursery seedlings. Small numbers of mycorrhizas may often produce a disproportionately large number of fruitbodies.

Fruitbody observations support the view that this is a very common, non-specific, 'early stage' fungus, found in a wide range of habitats in many parts of the world.

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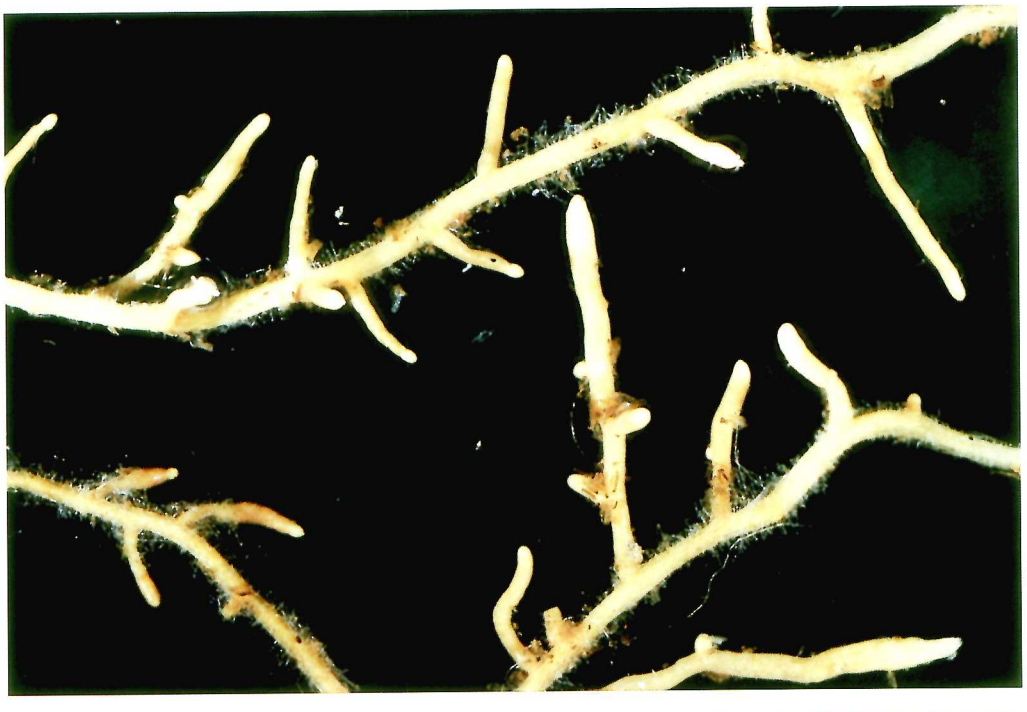
Associated trees:
Betula pendula
Picea sitchensis

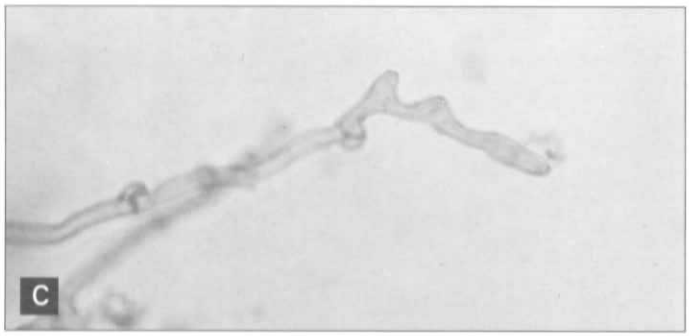
Class: **BASIDIOMYCOTINA**
Order: **AGARICALES**
Family: **TRICHOLOMATACEAE**

11

Laccaria tortilis

([Bolt.] S.F. Gray) Cooke

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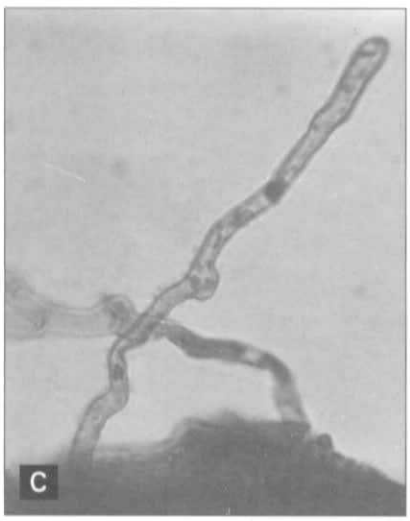
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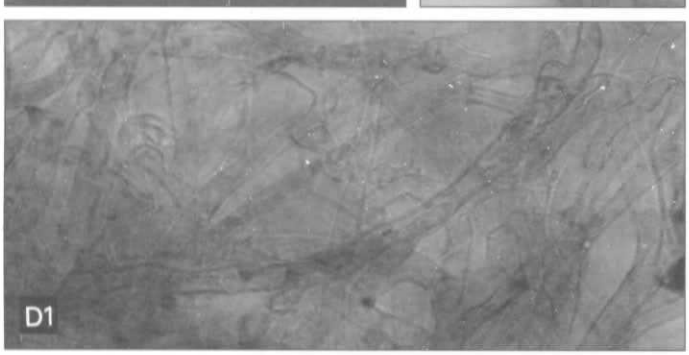
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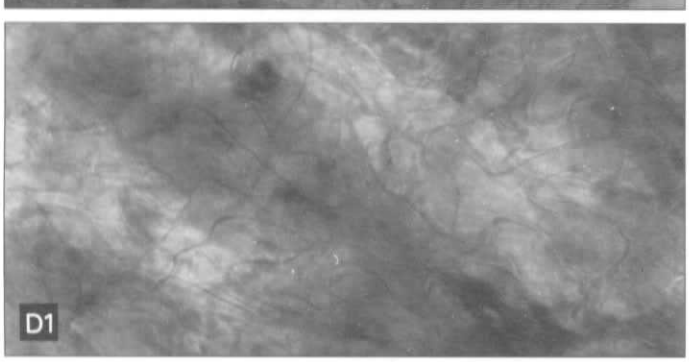
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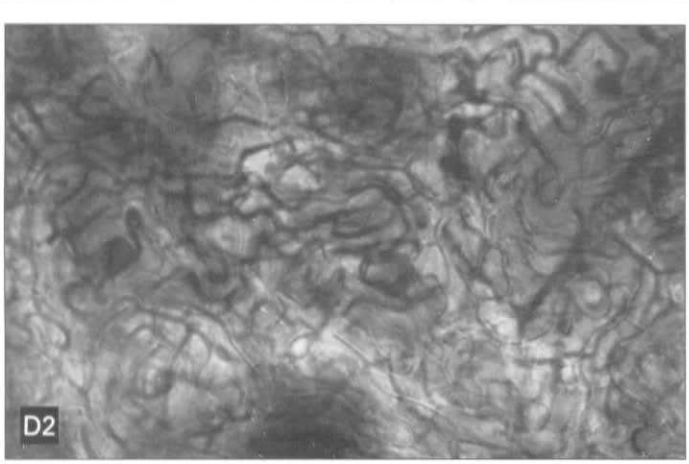
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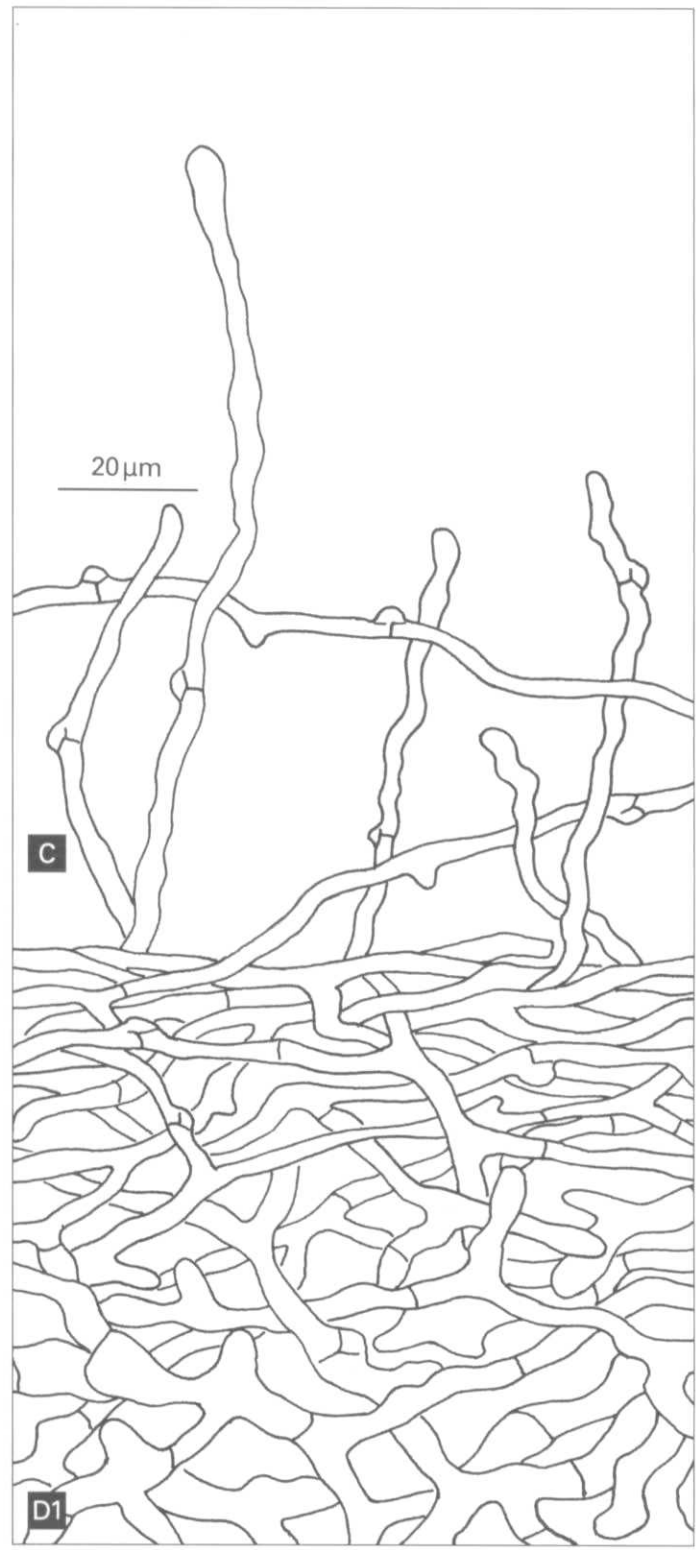
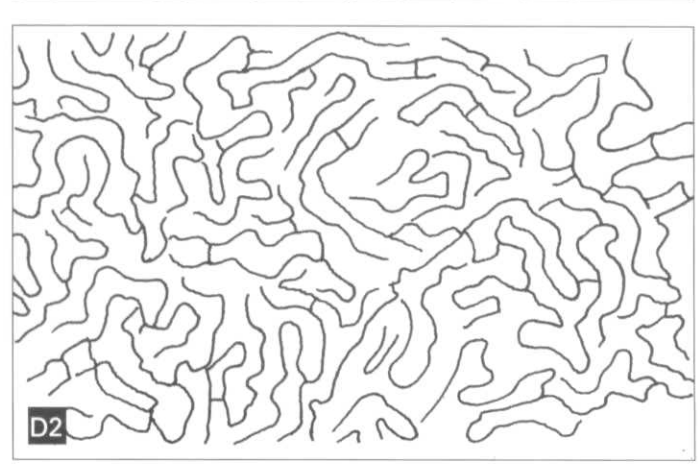

20 µm
C
D1


D2

Laccaria tortilis

([Bolt.] S.F. Gray) Cooke

Identification:
Synthesis, fruitbody links

Macroscopic appearance

Mycorrhizas are fairly long, with frequent, irregularly spaced, short branches. The main axis is <7 mm in length and <0.5 mm in diameter.

Loose hyphae can be found associated with the mantle surface. These hyphae are often of a determinant length (0.1–0.2 mm) and can be sufficiently numerous to form a fringe of hyphae just visible under the dissecting microscope.

Mycorrhizas are white to fawn when young, darkening with age. All mycorrhizas retain an opaque milk-white lustre.

Microscopic appearance

A. *Strands:* not observed.
B. *Sclerotia:* not observed.
C. *Mantle edge:* loosely formed becoming compacted in older mycorrhizas. Emanating hyphae: 2.5–3.5 µm in diameter, with abundant large, irregularly formed clamp-connections and elbow-like protrusions. Fringe hyphae contain 2–3 septa, invariably with clamp-connections. They are often distinctly flexuous, with swollen hyphal tips.
Specialised elements: not observed.
D. *Mantle:* 10–30 µm in depth.
D1. *Surface:* a net prosenchyma of loosely interwoven hyphae 2.5–5.0 µm in diameter, with occasional clamp-connections. Hyphae in the young mantle stain strongly blue in lactophenol cotton blue. Hyphae broaden (<8 µm in diameter) and interlock more closely with age to form a net synenchyma.
D2. *Inner:* a net synenchyma of tortuous lobate cells, <25 µm in length and <5 µm in diameter.

Distinguishing features

Although possessing a similar mantle structure to those of *Laccaria proxima* (Boud.) Pat. and *Laccaria amethystea* (Bull.: Mérat) Murr., this mycorrhiza can be distinguished by the characteristic microscopic appearance of the fringe hyphae. Like *L. proxima,* care should be taken to distinguish it from very young mycorrhizas of *Thelephora terrestris* (Ehrh.) Fr. which have not developed a compact mantle surface.

Ecology

Mycorrhizas have frequently been recorded on birch and spruce seedlings of 1–15 years of age growing on brown earth sites near Edinburgh. Our observations suggest that small numbers of mycorrhizas are often responsible for a disproportionately large number of fruitbodies.

Fruitbody observations suggest that this fungus is also associated with *Pinus* spp.

References

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THOMAS, G.W. & JACKSON, R.M. 1982. Scanning electron microscopy of sheathing mycorrhizas of Sitka spruce. *Trans. Br. mycol. Soc.*, **79,** 31–39.

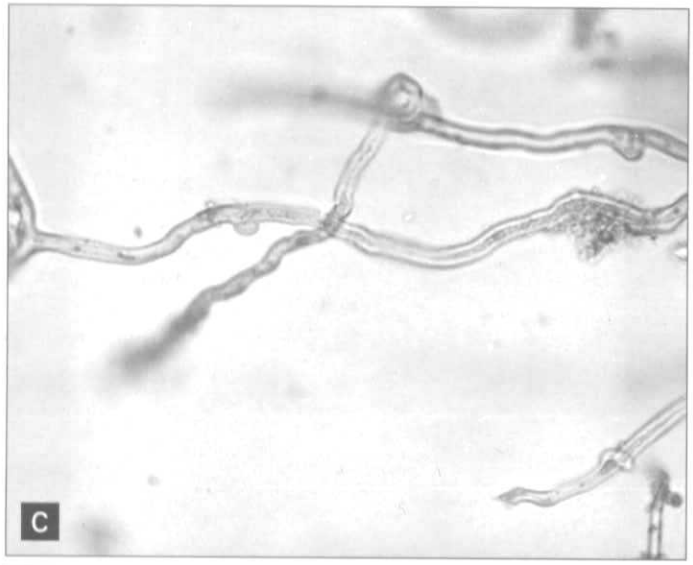
Associated trees:
Betula pendula
Picea sitchensis
Pseudotsuga menziesii

Class: **BASIDIOMYCOTINA**
Order: **AGARICALES**
Family: **CORTINARIACEAE**

12

Inocybe petiginosa

(Fr.: Fr.) Gillet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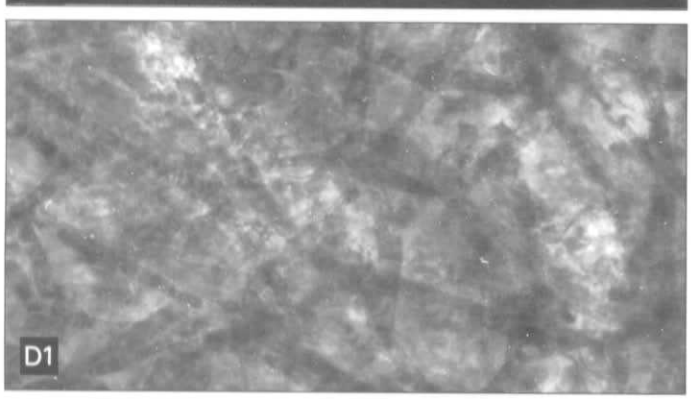
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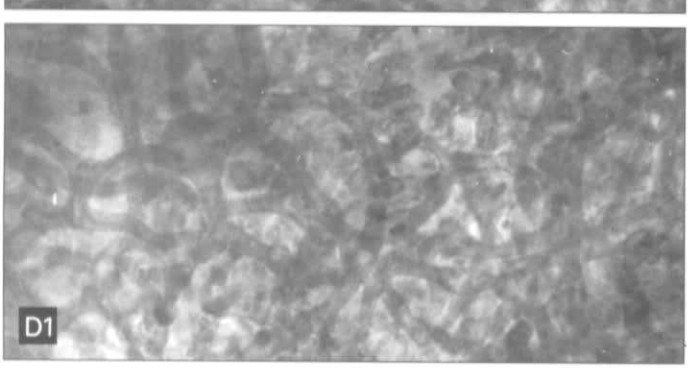
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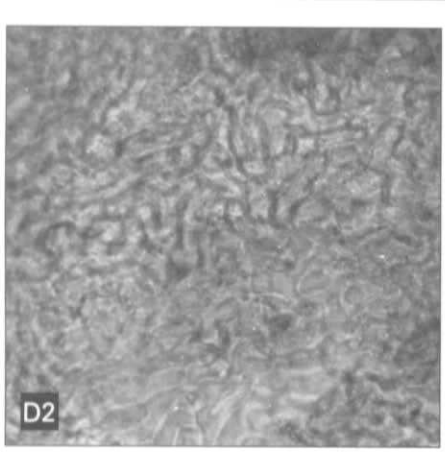
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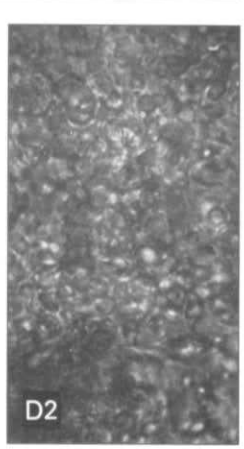
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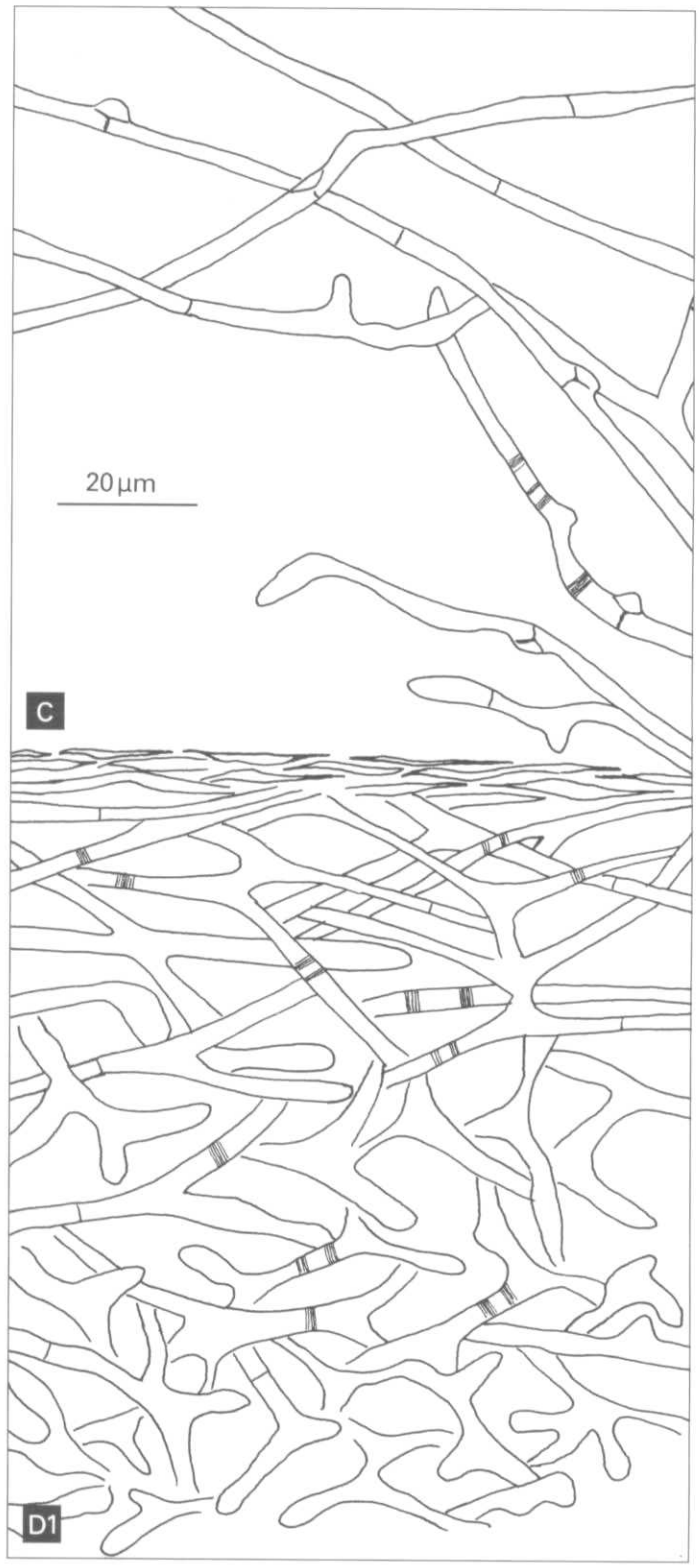
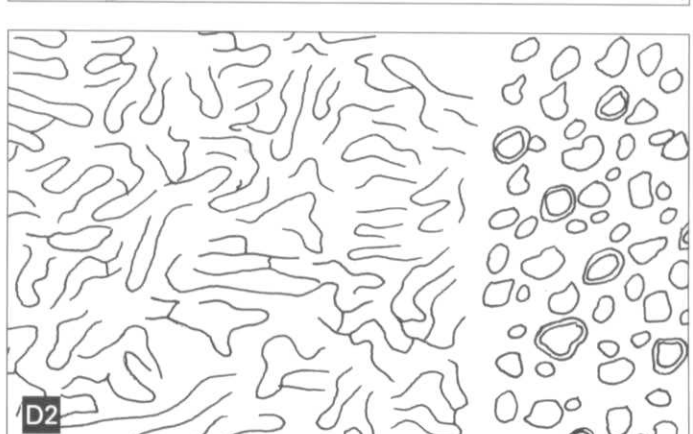

20 µm
C
D1


D2

Inocybe petiginosa

(Fr.: Fr.) Gillet

Identification:
Fruitbody links

Macroscopic appearance

Mycorrhizas are short and stubby, with a frequent, irregular branching pattern. The main axis is <3 mm in length and <0.6 mm in diameter.

The mantle surface is conspicuously smooth and shiny. Loose, straggly hyphae are occasionally found, often around the base of the mycorrhiza.

Mycorrhizas are white when young, slowly browning with age.

Microscopic appearance

A. *Strands:* not observed.
B. *Sclerotia:* not observed.
C. *Mantle edge:* smooth but not compact, consisting of distinctly adpressed hyphae.
   Emanating hyphae: 2–3 µm in diameter, with abundant clamp-connections and elbow-like protrusions. Hyphae found close to the mantle surface are sometimes distinctly banded.
   Specialised elements: not observed.
D. *Mantle:* 20–40 µm in depth.
   D1. *Surface:* a net prosenchyma of distinctly arranged, shortened, dichotomously branched hyphae, 2–5 µm in diameter, which stain strongly blue in lactophenol cotton blue. Like the emanating hyphae, some of these mantle hyphae are banded.
   As the mantle ages, these hyphal cells become shorter and broader, and interlock more closely.
   D2. *Inner:* a net synenchyma of tortuous, interwoven cells, <15 µm in length and <5 µm in diameter. These cells are also characterised by large numbers of oily globules, which make the lower mantle features difficult to examine.

Distinguishing features

Like other *Inocybe* spp. examined, *I. petiginosa* can be distinguished macroscopically by the short, stubby, shiny, white mycorrhizas, and microscopically by the distinctive appearance of the mantle surface.

Although separation at species level is difficult, this mycorrhiza can be distinguished from those of *Inocybe lacera* (Fr.) Quél. and *Inocybe lanuginella* (Schroet.) Konrad & Maublanc by the banding of the surface hyphae and by the lack of distinctly 'open' clamp-connections with strongly staining septa.

Ecology

This is a very commonly occurring, but never abundant, mycorrhiza of containerised glasshouse and nursery seedlings. Our observations suggest that small numbers of mycorrhizas are responsible for a disproportionately large number of fruitbodies.

Fruitbody observations suggest that this fungus is associated with a wide range of host trees but, because of its small size, it may often be overlooked.

References

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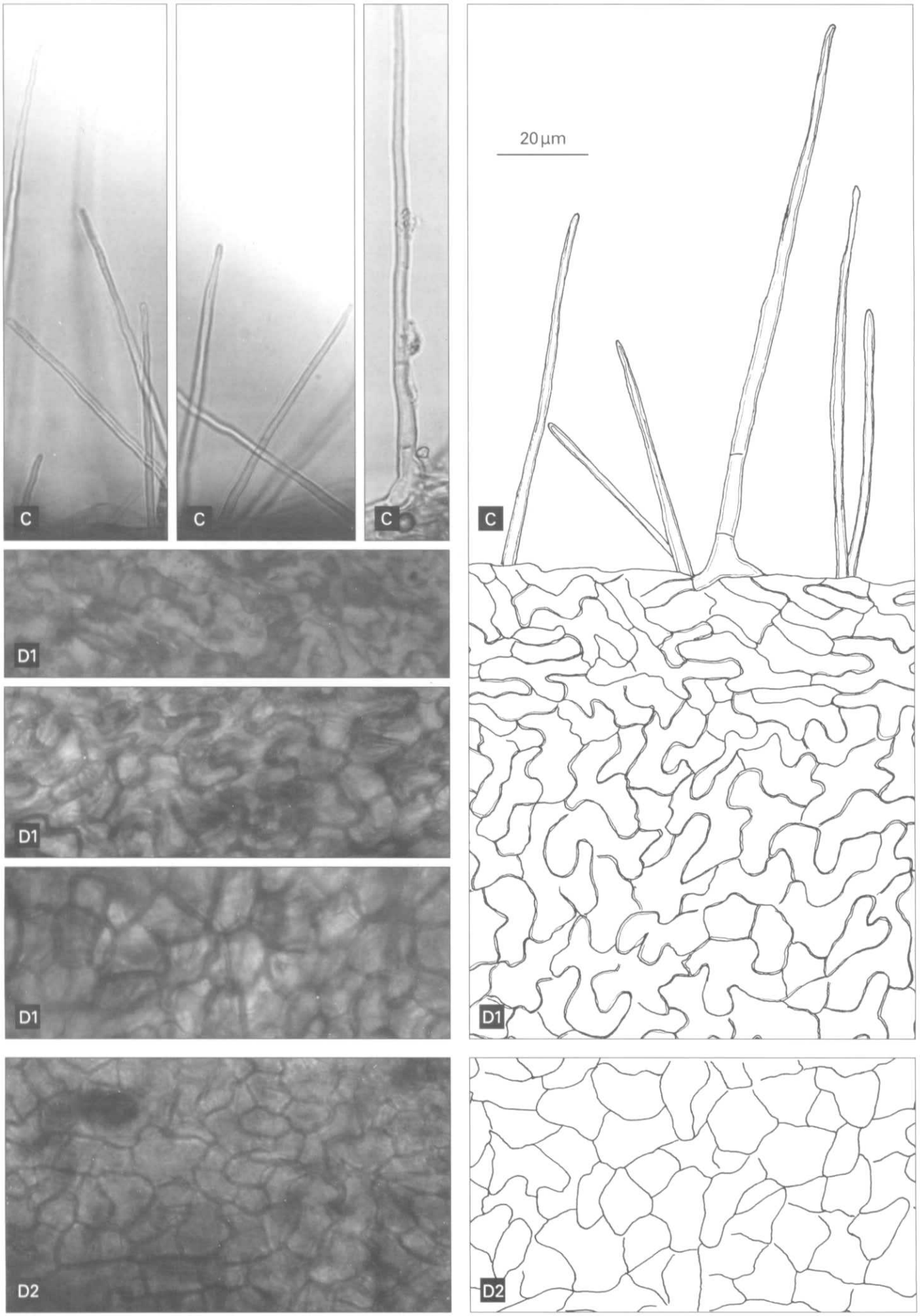
Associated trees:
Betula pendula
Picea sitchensis
Pseudotsuga menziesii
Quercus robur

Class: **ASCOMYCOTINA**
Order: **TUBERALES**
Family: **EUTUBERACEAE**

13

Tuber sp.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C
C
D1
D1
D1
D2
20 µm
C
D1
D2

Tuber sp.

Identification:
Literature descriptions

Macroscopic appearance

Mycorrhizas are short and stubby, with frequent, irregular, often short branches. The main axis is <5 mm in length and <0.5 mm in diameter.

The mantle surface is smooth and shiny but is often obscured by a dense covering of setae just visible under the dissecting microscope.

Mycorrhizas are buff (52) when young, rapidly darkening to chestnut (23), usually with a distinctly paler coloured tip.

Microscopic appearance

A. *Strands:* not observed.
B. *Sclerotia:* not observed.
C. *Mantle edge:* smooth and compact.
Specialised elements: setae are straight, unbranched, slightly thick-walled and extend up to 100 µm from the mantle surface. The setae are 4–5 µm in diameter at the base, tapering to an acute tip. They contain 1–3 septa, with one usually immediately above the base.
D. *Mantle:* 20–35 µm in depth.
D1. *Surface:* an irregular, interlocking synenchyma often varying to an irregular non-interlocking synenchyma composed of thick-walled cells 5–20 µm in diameter. The mantle surface is often obscured by the broad bases of the setae when there is a dense covering.
D2. *Inner:* an irregular non-interlocking synenchyma of cells 5–20 µm in diameter with non-thickened cell walls.

Distinguishing features

This mycorrhiza is readily distinguished by features of setae and mantle surface. Mycorrhizas with similar setae and thick synenchymous mantles have been described by Fontana and Centrella (1967) (*Tuber albidum* Pico. on *Pinus pinea* L., *Quercus petraea* Liebl. and *Corylus avellana* L.), Palenzona and Fontana (1978) (*Tuber magnatum* Pico. on *Quercus pubescens* Willd.), Chu-Chou and Grace (1983) (*Tuber* sp. on *Pseudotsuga menziesii*), and Voiry (1981) (*T. albidum* on *Fagus sylvatica* L.).

Ecology

These mycorrhizas have been recorded on several hosts of 1–10 years of age, growing in a wide range of soils including brown earths, nursery soils and coal wastes.

Fruitbody observations would also suggest that these mycorrhizas remain common on *Corylus, Fagus* and *Quercus* trees up to 25 years of age, particularly those growing in calcareous soils.

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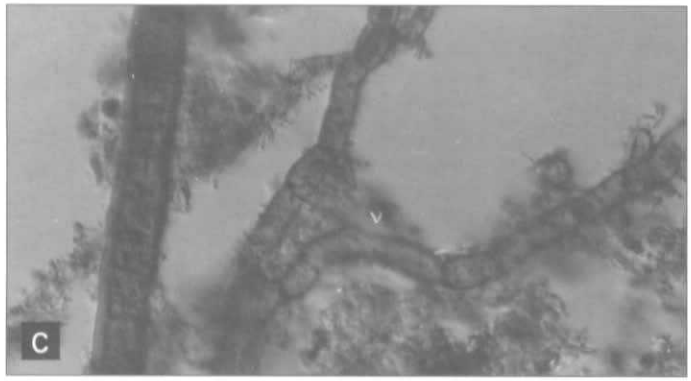
Associated trees:
Betula pendula

Class: **ASCOMYCOTINA**
Order: **TUBERALES**
Family:

14

Type: ITE.4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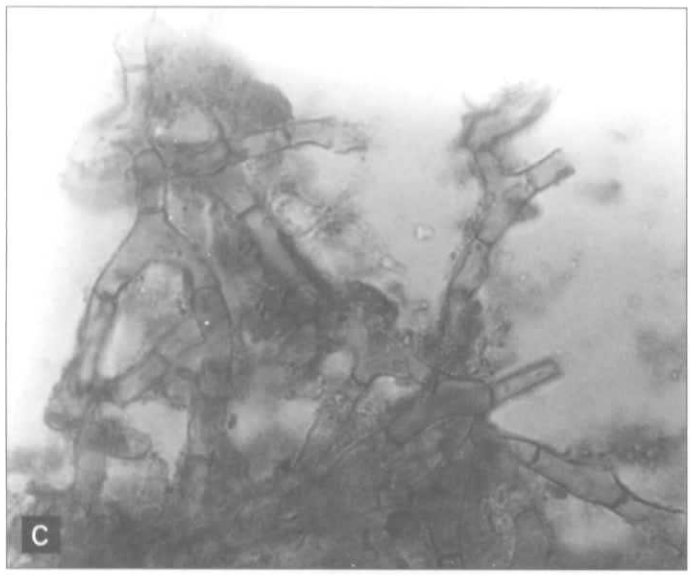
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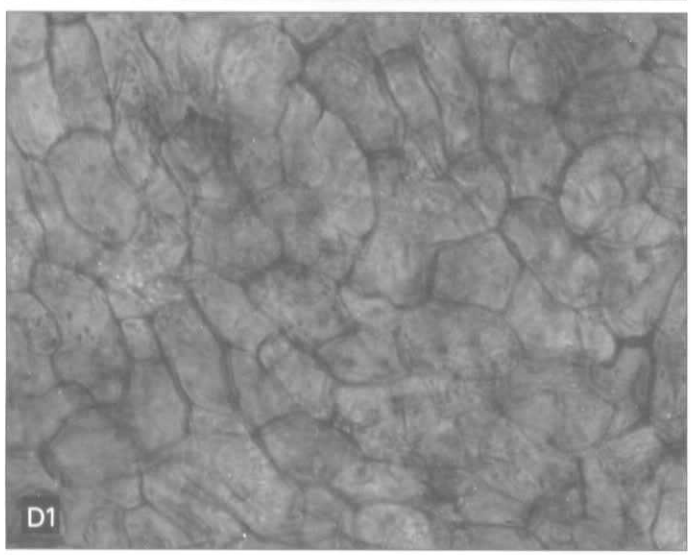
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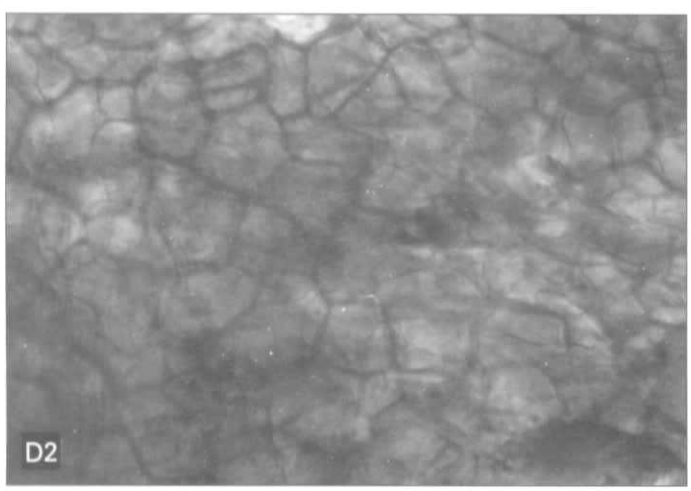
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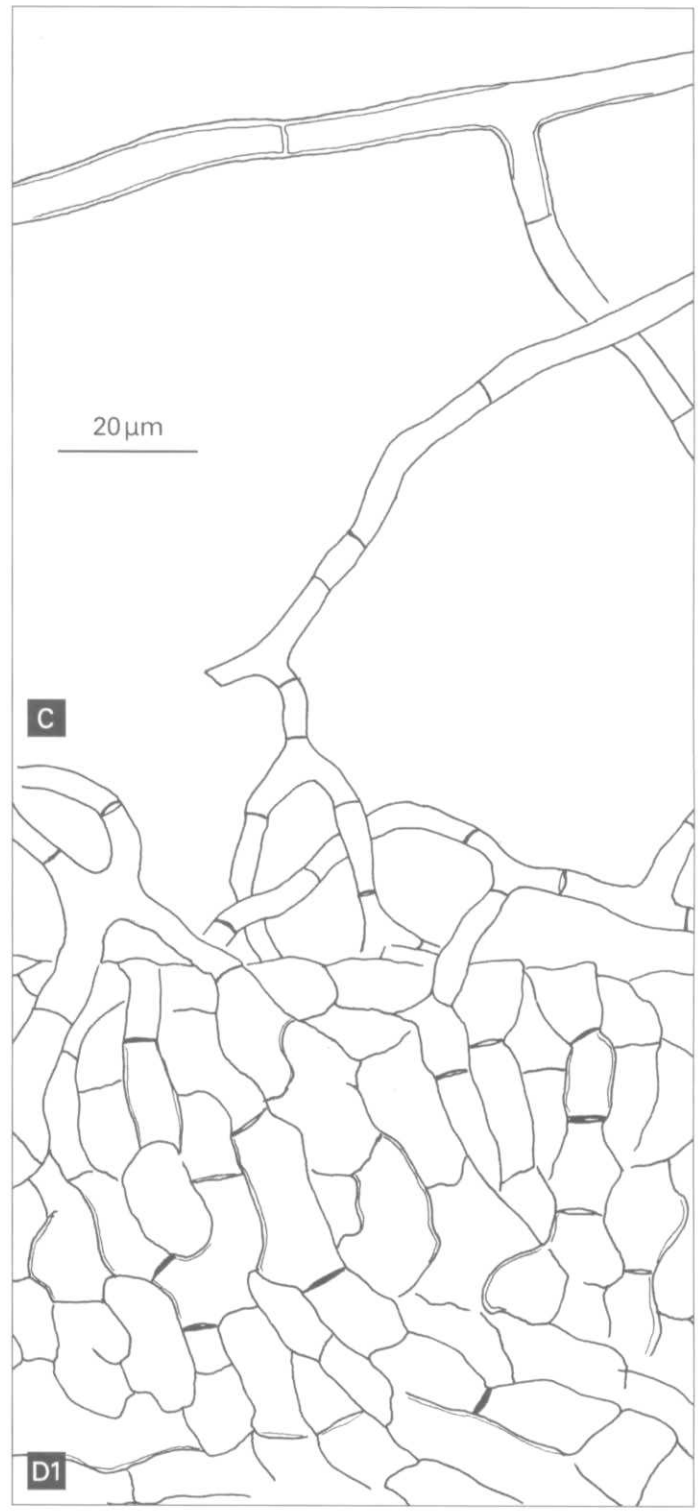
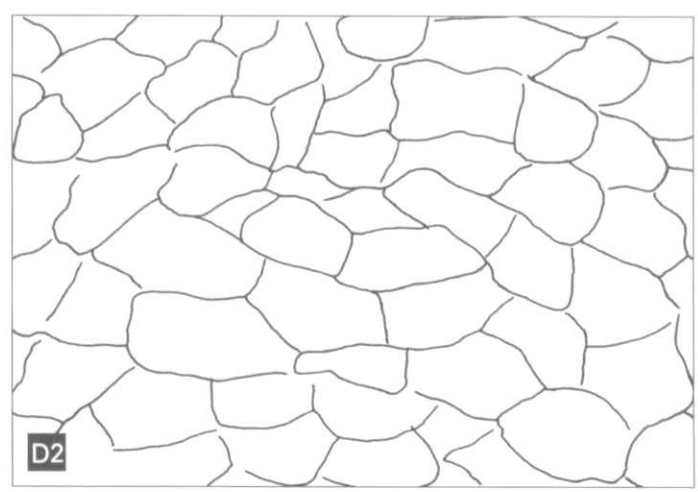

20 µm
C
D1


D2

Type: ITE.4

Identification:
Literature descriptions

Macroscopic appearance

Mycorrhizas are short, with bluntly rounded tips and frequent, irregularly spaced branches. The main axis is <5 mm in length and <0.5 mm in diameter.

The mantle surface is reticulate and shiny. Loose, straggly hyphae are frequently found emanating from the mantle surface.

Mycorrhizas are white when young, changing to clay-buff (32) with age.

Microscopic appearance

A. *Strands:* not observed.
B. *Sclerotia:* not observed.
C. *Mantle edge:* compact and uneven.
Emanating hyphae: loose hyphae are 4–10 µm in diameter, septate and infrequently branched. Those closely associated with the mantle are shorter celled (<20 µm in length), distinctly septate, and highly branched. These hyphal cells are often inflated, narrowing at the septa (3–7 µm in diameter).
Specialised elements: not observed.
D. *Mantle:* 15–30 µm in depth.
D1. *Surface:* a compact structure of broad hyphal elements forming a net synenchyma or a non-interlocking irregular synenchyma. These cells are 4–20 µm in diameter, and may have slightly thicker-walled septa.
D2. *Inner:* a compact structure of rounded or angular, isodiametric, cells, 4–20 µm in diameter, which form a regular synenchyma or a non-interlocking irregular synenchyma.

Distinguishing features

This mycorrhiza is macroscopically inconspicuous, but microscopic features of the emanating hyphae suggest that this fungus is an ascomycete. The additional feature of a fairly thick synenchymous mantle suggests that it may be placed within the *Tuberales*. The description resembles that given by Fontana and Centrella (1967) for the hypogeous ascomycete *Genea Klotzschii* Berk. & Br. on *Quercus petraea* Liebl.

Ecology

Mycorrhizas have been found in abundance on one year old *Betula* spp. seedlings growing on a brown earth site near Edinburgh.

Reference FONTANA, A. & CENTRELLA, E. 1967. Ectomycorrhizae produced by hypogeous fungi. *Allionia,* **9,** 113–118.

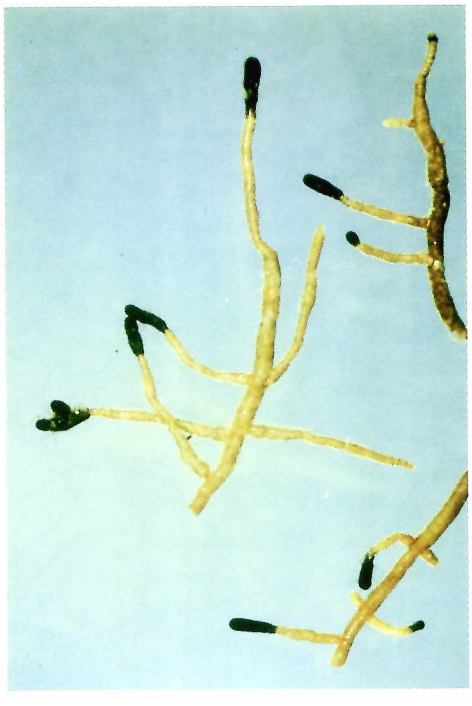
Associated trees:
Betula pendula
Picea sitchensis
Pinus sylvestris

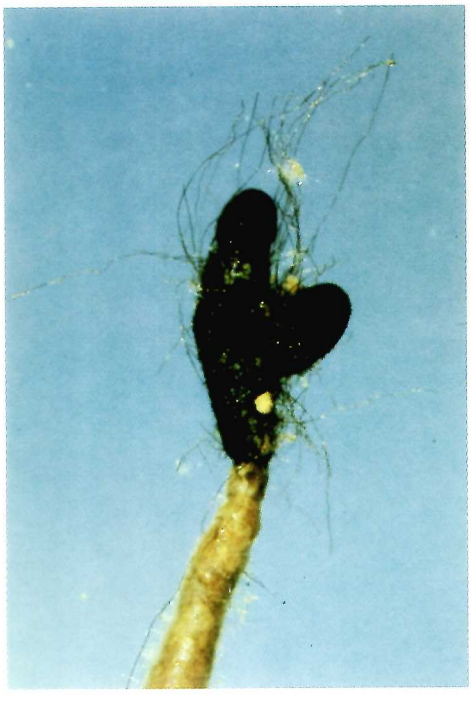
Class: **DEUTEROMYCOTINA**
Order:
Family:

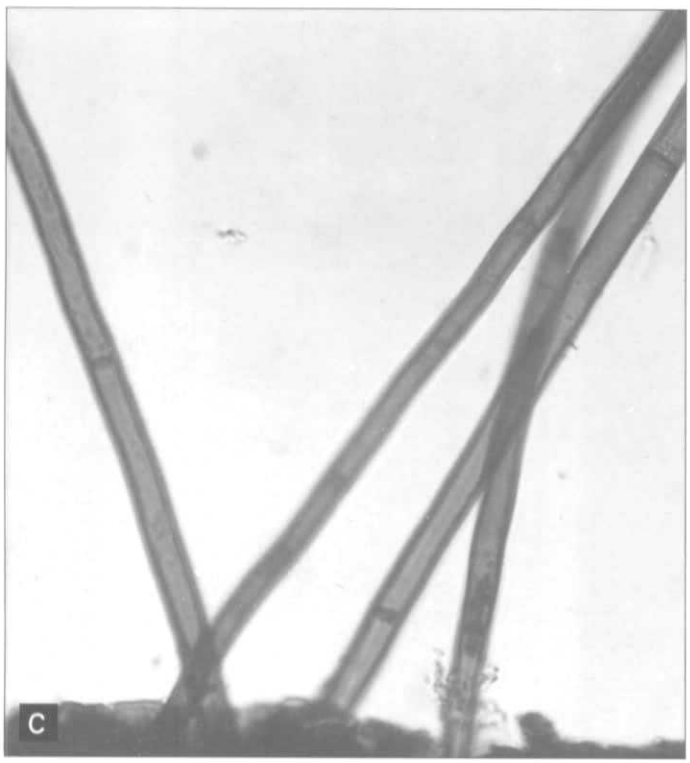
15

Cenococcum geophilum

Fr.



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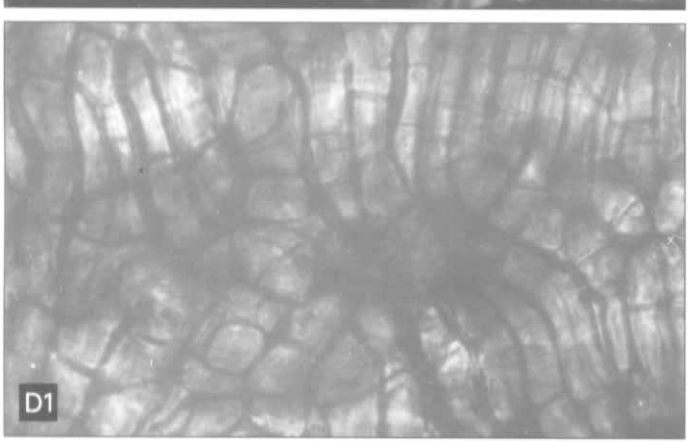
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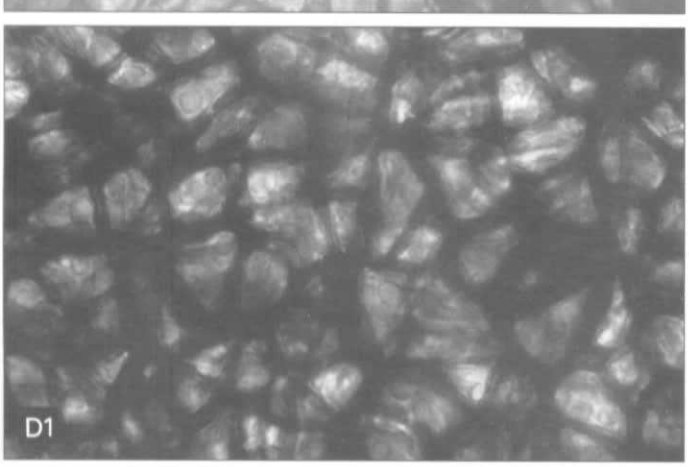
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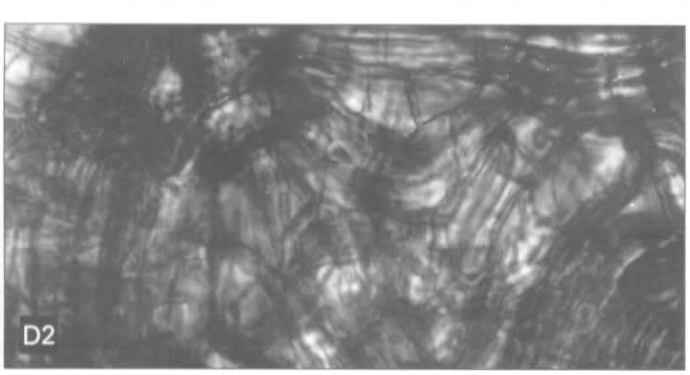
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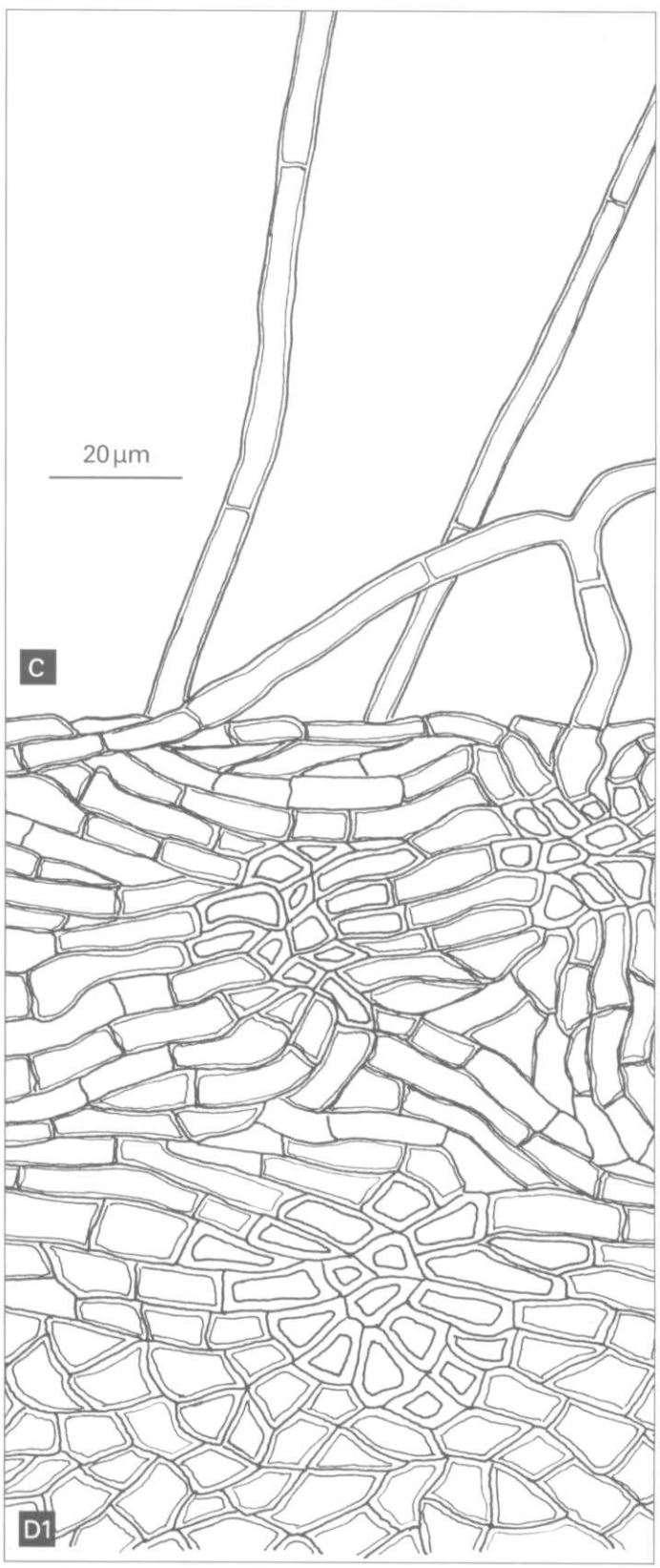
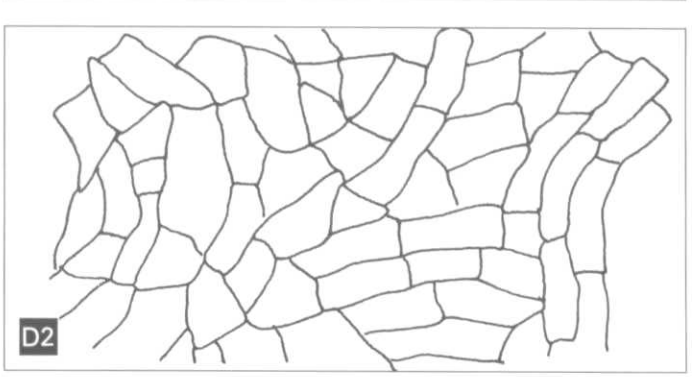

20 µm
C
D1


D2

Cenococcum geophilum

Fr.

Identification:
Synthesis, literature descriptions

Macroscopic appearance

Mycorrhizas are short and often club-shaped. They are invariably single, but may occasionally produce one or two short branches. The main axis is <2.5 mm in length and <0.4 mm in diameter.

Thick, black hyphae are frequently observed radiating from the mantle surface. These hyphae disappear as the mycorrhiza ages.

Mycorrhizas are black when young, remaining black with age.

Microscopic appearance

A. *Strands:* not observed.
B. *Sclerotia:* present but rarely observed in direct association with the mycorrhizas, as any hyphal attachment is very fragile and easily broken during the removal of adhering soil. Sclerotia are hard, smooth and spherical, <2 mm in diameter and black in colour.
Chilvers (1968) has described the rind cells as angular, isodiametric cells, 5–8 µm in diameter.
C. *Mantle edge:* compact and uneven.
Emanating hyphae: 4–6 µm in diameter, straight, and invariably broken because of their fragile nature. These hyphae are brown in colour, distinctly septate, with thickened cell walls and sometimes finely verrucose. They may be absent in older mycorrhizas.
Specialised elements: not observed.
D. *Mantle:* 10–25 µm in depth.
D1. *Surface:* typically a net synenchyma of heavily thickened cells 4–8 µm in diameter and <25 µm in length. These cells radiate from clusters of isodiametric, even thicker-walled cells.
A second form is also encountered where isodiametric, heavily thickened cells form a regular synenchyma. These cells are also arranged in radiating clusters.
D2. *Inner:* a net synenchyma of isodiametric or slightly elongate, non-thickened cells 3–8 µm in diameter.

Distinguishing features

This is perhaps the most widely described mycorrhiza, black in colour with characteristic emanating hyphae and mantle surface features. However, care should be taken to examine microscopic features, as other brown/black mycorrhizas (eg ITE.5) may appear macroscopically similar to older mycorrhizas of *C. geophilum,* where the emanating hyphae are absent.

Ecology

Mycorrhizas are ubiquitous on seedlings and mature trees, but usually occur in small numbers and rarely dominate a root system. Several authors consider this fungus to be drought-tolerant.

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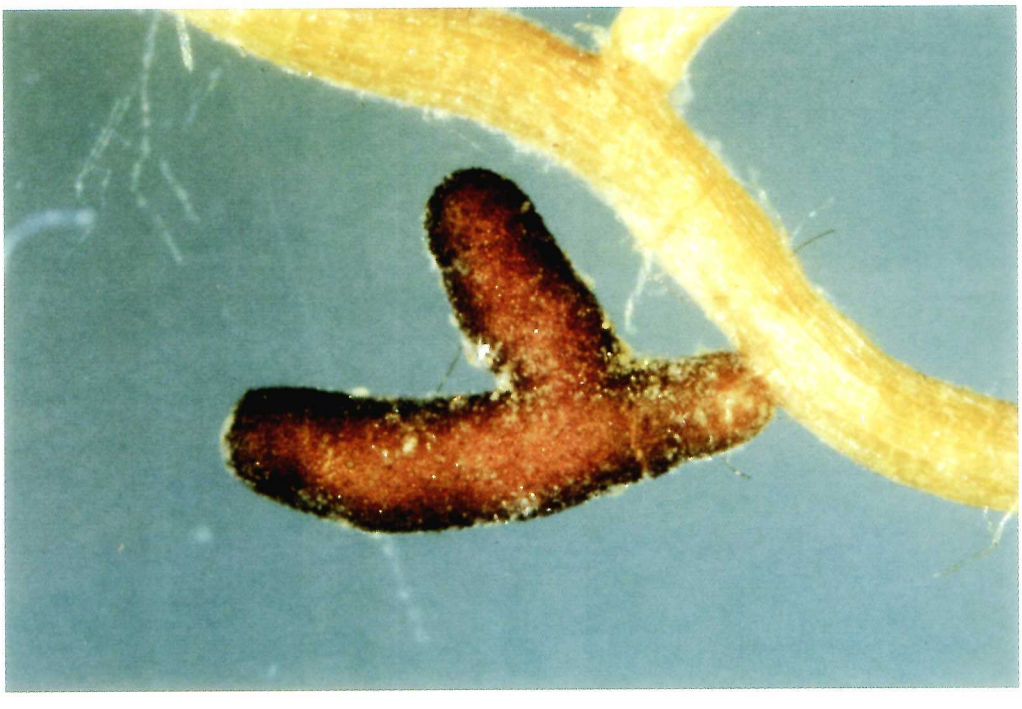
VOIRY, H. 1981. Classification morphologique des ectomycorrhizes du chêne et du hêtre dans le nord-est de la France. *Eur. J. For. Path.,* **11,** 284–299.

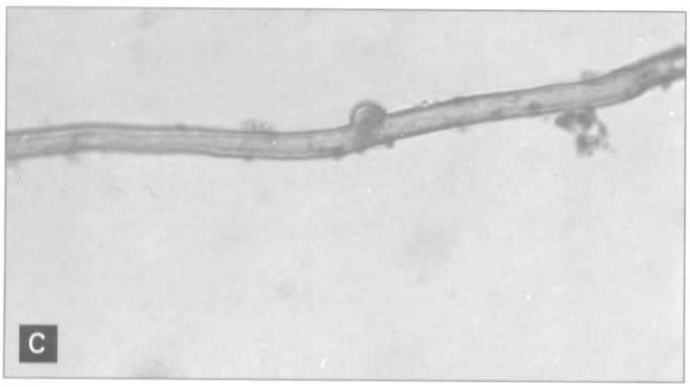
Associated trees:
Betula pendula
Picea sitchensis

Class: **BASIDIOMYCOTINA**
Order:
Family:

16

Type: ITE.5

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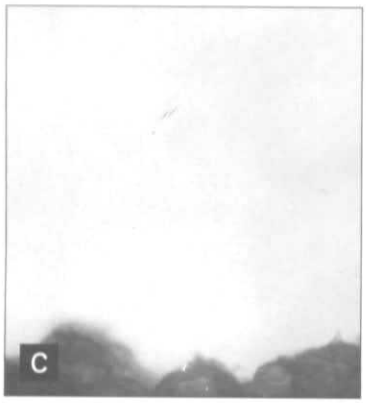
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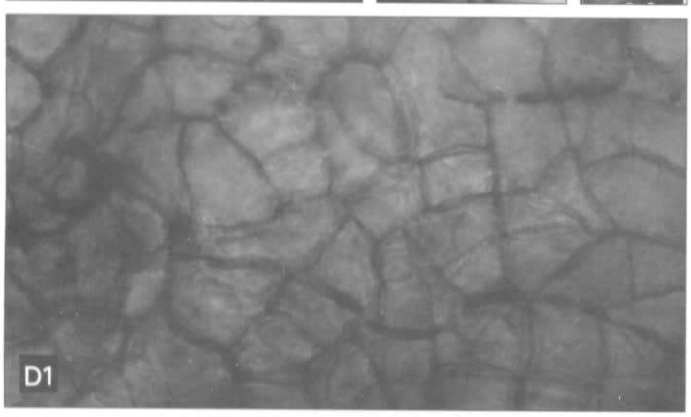
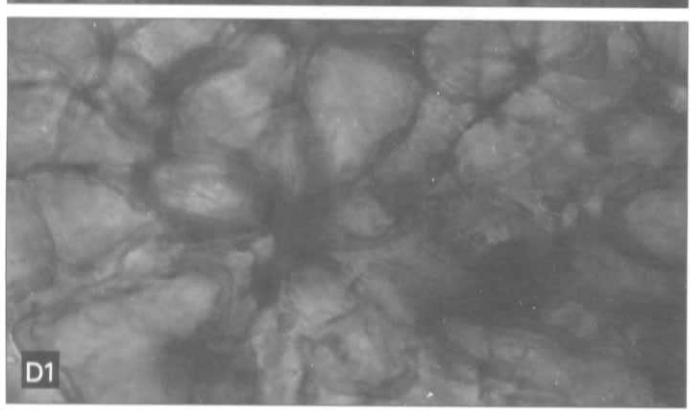
C


D1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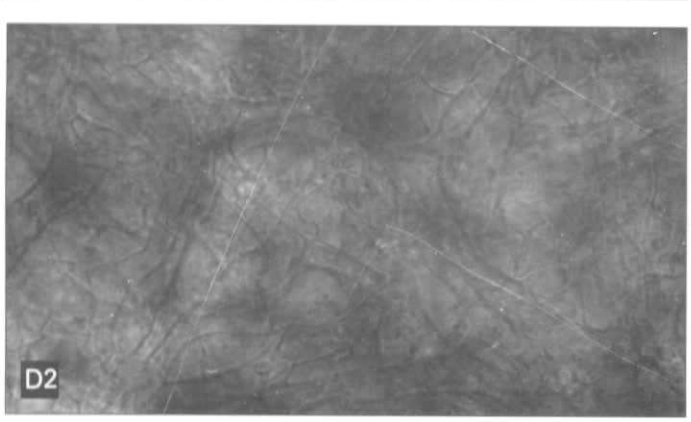
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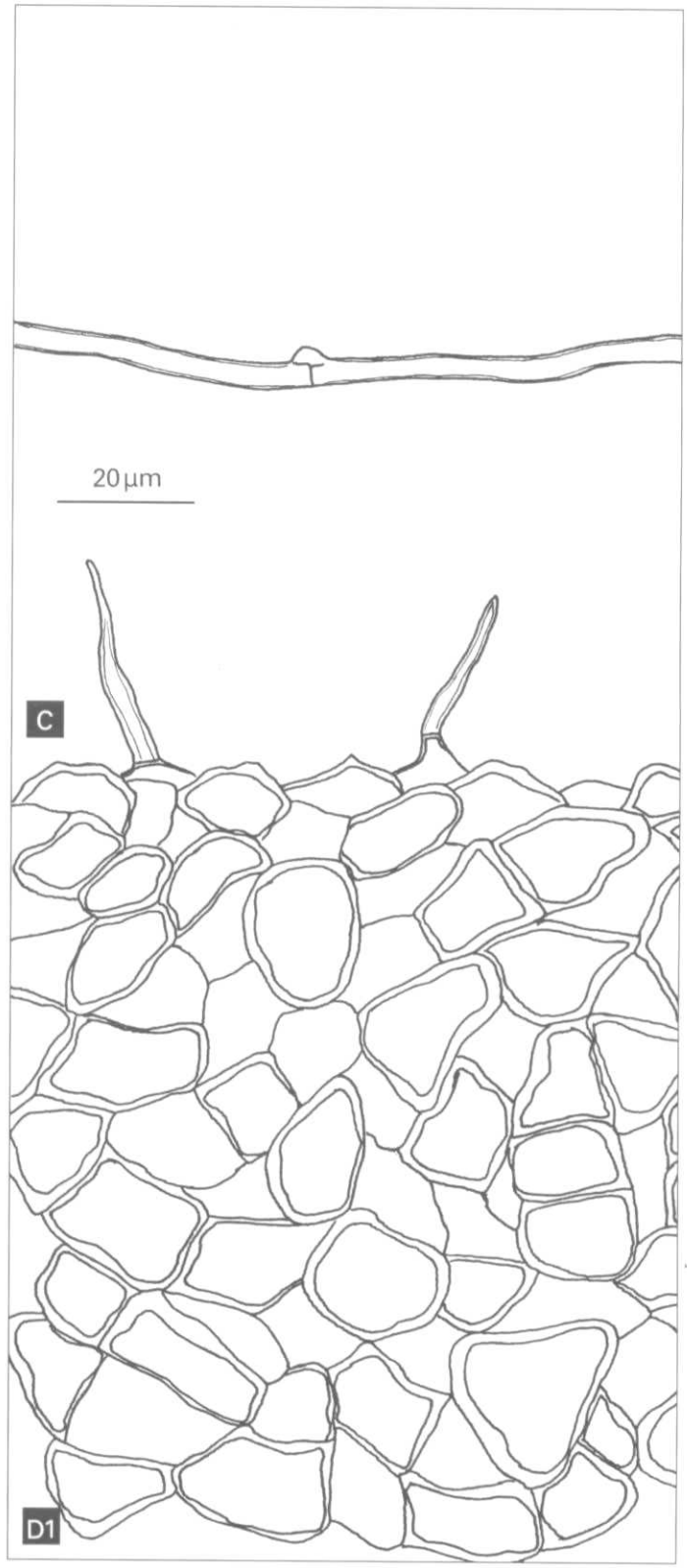
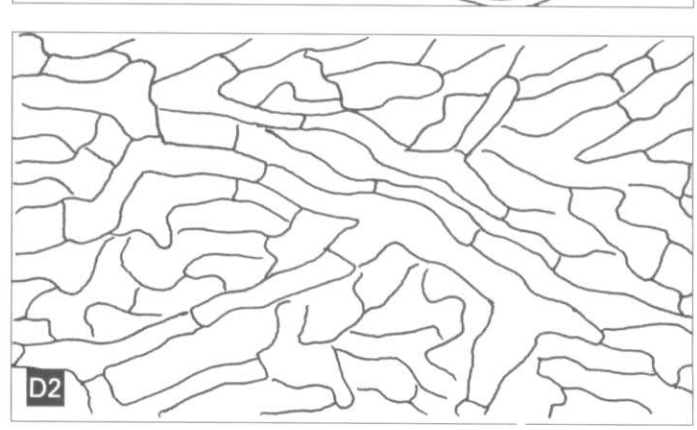

20 µm
C
D1


D2

Type: ITE.5

Identification:

Macroscopic appearance

Mycorrhizas are short, stubby and blunt-ended, with a frequent, sometimes pinnate, branching pattern. The main axis is <6 mm in length and <0.6 mm in diameter.

The mantle surface is distinctly granular in appearance, with loose hyphae only rarely observed.

Mycorrhizas are blackish-bay (19) when young, changing to blackish cigar-brown (16) with age.

Microscopic appearance

A. *Strands:* not observed.
B. *Sclerotia:* not observed.
C. *Mantle edge:* compact and very uneven, composed of large cells which often possess a single, short projecting spine <2 µm in length.
Emanating hyphae: rarely found, but, when present, are yellow in colour, 3–5 µm in diameter, with clamp-connections.
Specialised elements: setae are regularly observed although not abundant, occurring singly or in clusters of 2–5. They are <50 µm in length, thick-walled, with the base (3–5 µm in diameter) gently tapering to a point.
D. *Mantle:* 20–30 µm in depth.
D1. *Surface:* a regular synenchyma of thick-walled, angular, often rounded cells 5–20 µm in diameter. As the mantle ages, these cells become larger, the thickened cell walls attaining 4 µm in diameter.
D2. *Inner:* a net synenchyma of hyphal elements 3–8 µm in diameter. The cell walls are not distinctly thickened.

Distinguishing features

This mycorrhiza is best distinguished by the combination of macroscopic appearance and distinctive characteristics of the mantle surface. However, care should be taken to examine microscopic features of the mantle surface as older mycorrhizas of *Cenococcum geophilum* Fr. may appear macroscopically similar. At present, we have no clues as to its identity.

Ecology

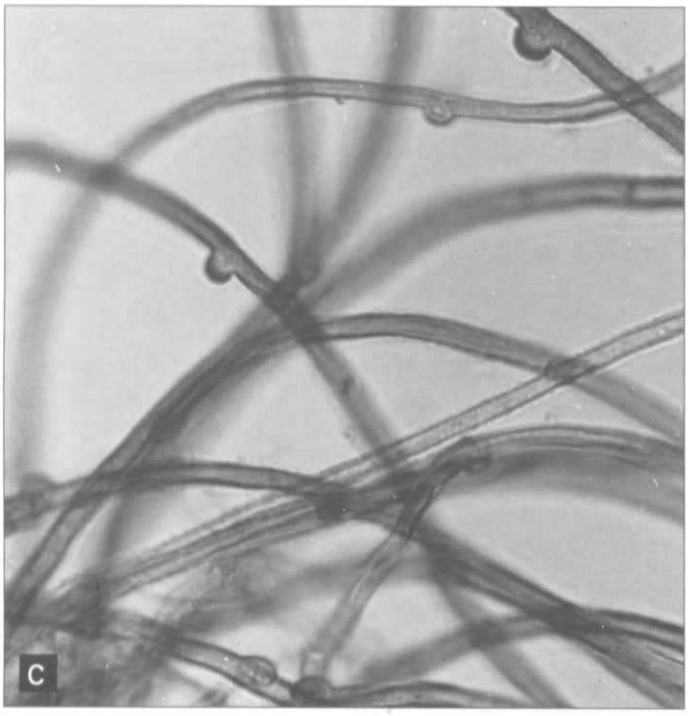
Mycorrhizas have frequently been recorded on *Picea* and *Betula* spp. seedlings of 2–10 years of age, growing in agricultural and forest brown earth soils.

Associated trees:
Picea sitchensis
Pinus sylvestris
Pseudotsuga menziesii

Class: **BASIDIOMYCOTINA**
Order:
Family:

17

Type: ITE.6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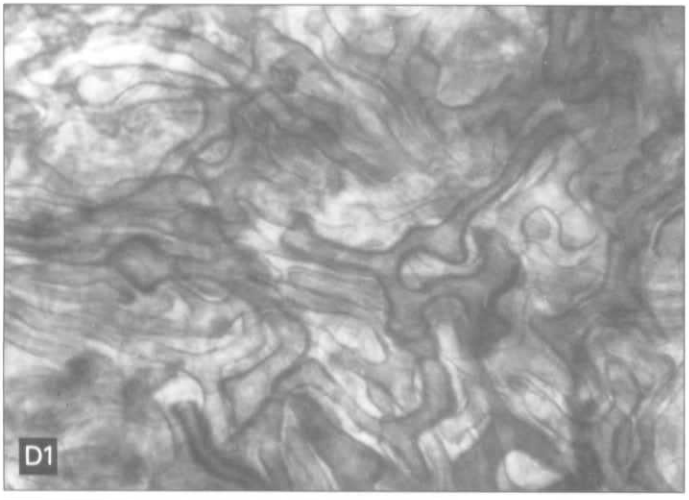
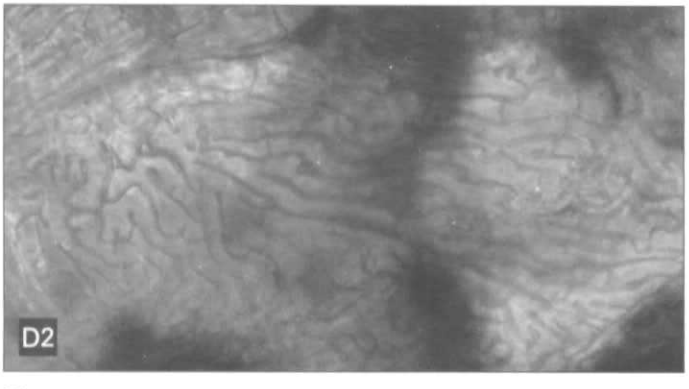

D1


D1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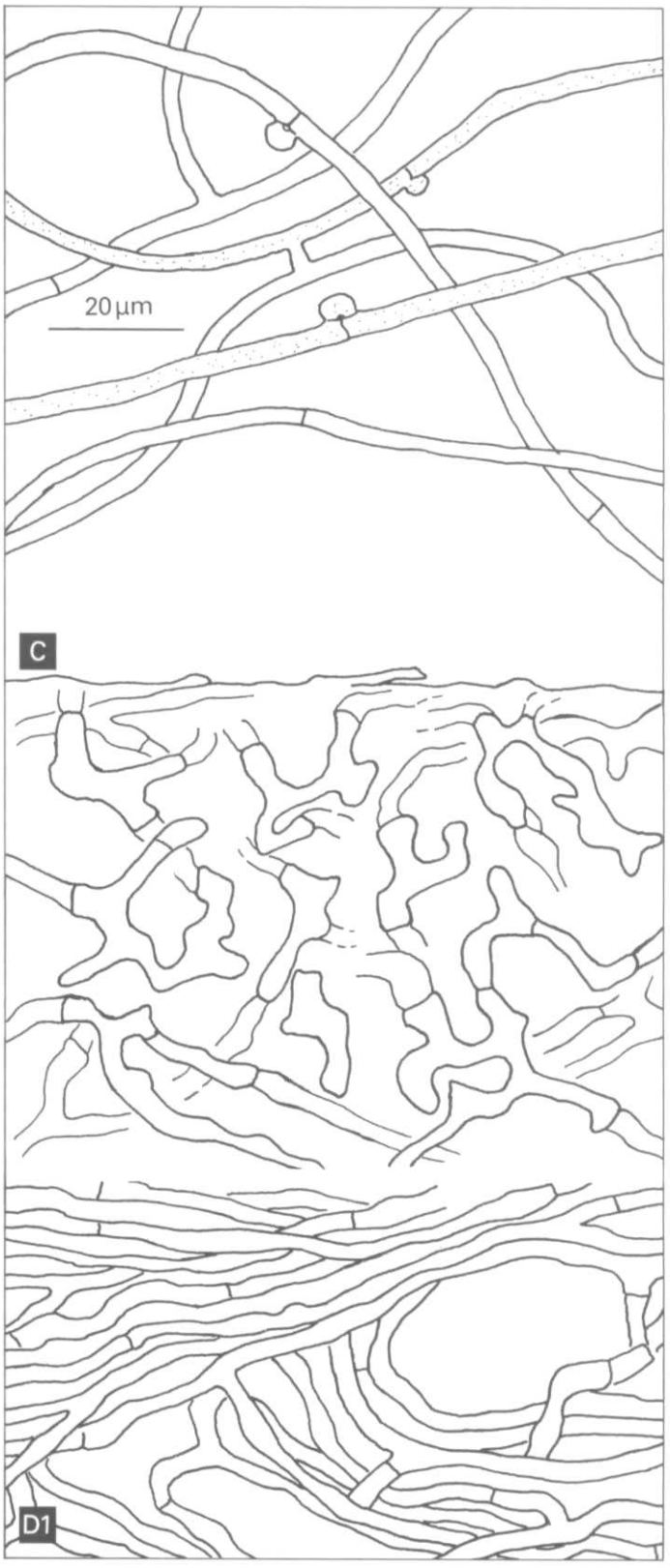
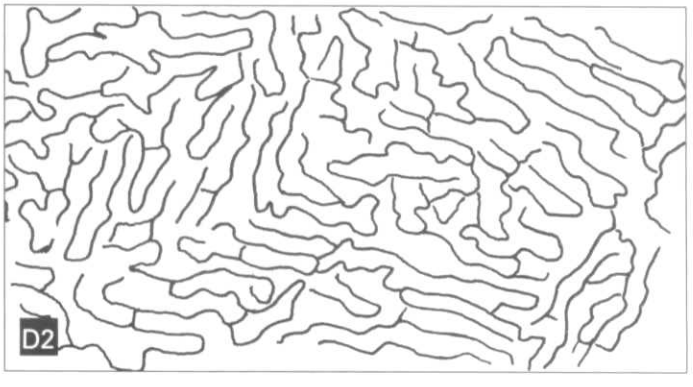

20 µm
C
D1


D2

Type: ITE.6

Identification:

Macroscopic appearance

Mycorrhizas are fairly long and sinuous, with frequent irregularly spaced branches. The main axis is <11 mm in length and <0.5 mm in diameter.

Dense abundant white hyphae are found surrounding the mantle. However, these hyphae are easily detached from the mantle, leaving the surface smooth and shiny and often appearing distinctly striate.

Mycorrhizas are buff (52) when young, changing to darker brown with age.

Microscopic appearance

A. *Strands:* not observed.
B. *Sclerotia:* not observed.
C. *Mantle edge:* smooth and fairly compact.
   Emanating hyphae: 2–4 µm in diameter, very finely verrucose, with prominent bulbous clamp-connections. These hyphae stain strongly lilac (79) or rose (39) in toluidine blue.
   Specialised elements: not observed.
D. *Mantle:* 8–15 µm in depth.
   D1. *Surface:* a net synenchyma of distinctly shaped hyphal elements (2–6 µm in diameter) which stain strongly rose (39) to vinaceous (76) in toluidine blue.
   As the mantle ages, a second form of net synenchyma may occasionally be found. In this instance, the configuration of the hyphae (1–3 µm in diameter) results in large elliptical spaces in the mantle surface. These holes may attain 40 µm in diameter.
   D2. *Inner:* a net synenchyma of tortuous cells 1–4 µm in diameter.

Distinguishing features

The staining properties of this mycorrhiza in toluidine blue, combined with the characteristics of mantle surface and associated hyphae, make this mycorrhiza easy to recognise when it is examined microscopically. At present, we have no clues as to its identity.

Ecology

Mycorrhizas have frequently been recorded on 2–6 year old *Picea sitchensis* (Bong.) Carr seedlings growing in nursery and forest brown earth soils. It has also been recorded in association with 10–15 year old *Pinus sylvestris* L. and *Pseudotsuga menziesii* (Mirb.) Franco growing in Glentress Forest, Peeblesshire, Scotland.

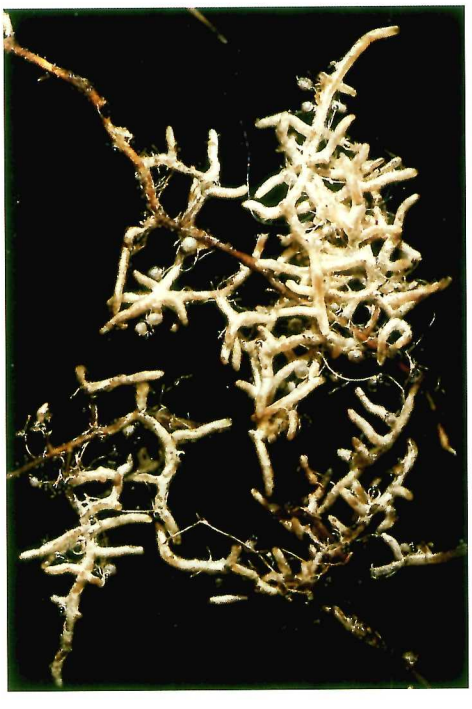
Associated trees:
Alnus glutinosa
Betula pendula
Betula pubescens
Picea sitchensis
Pinus contorta
Pinus sylvestris
Pseudotsuga menziesii

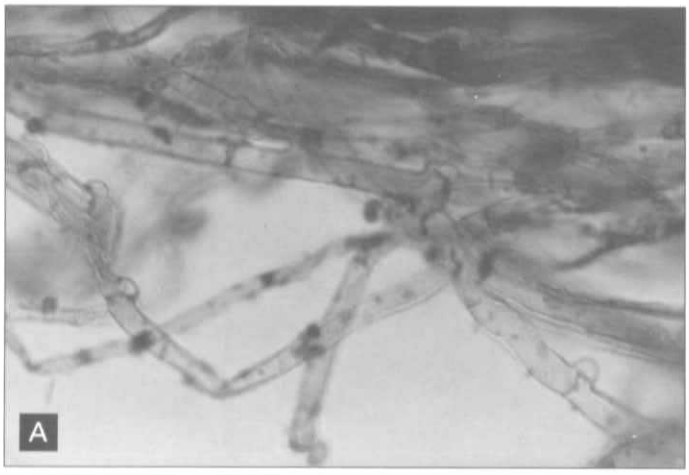
Class: **BASIDIOMYCOTINA**
Order: **BOLETALES**
Family: **PAXILLACEAE**

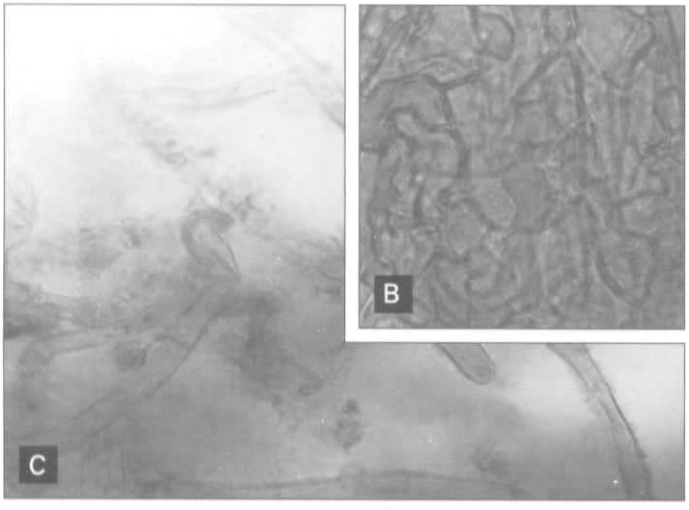
18

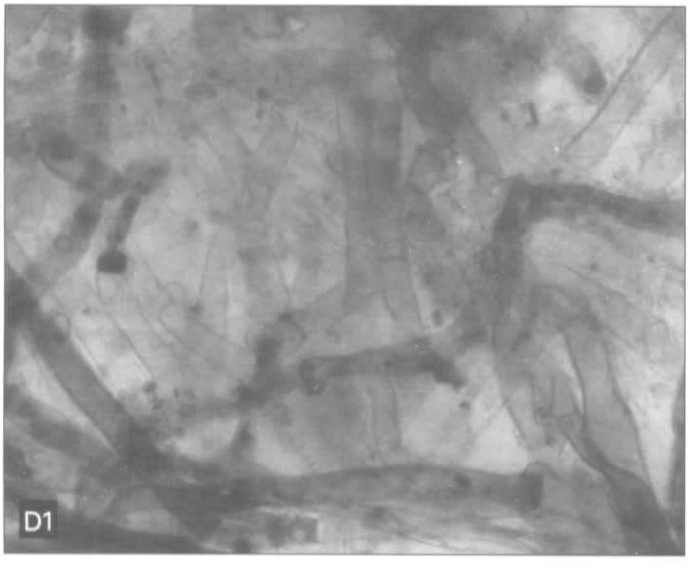
Paxillus involutus

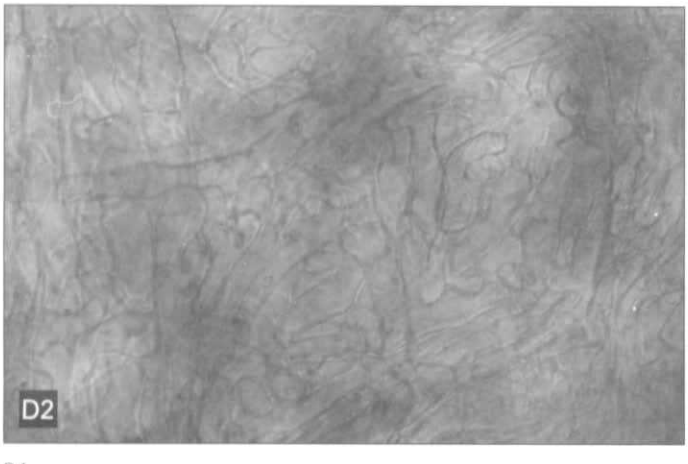
(Batsch) Fr.

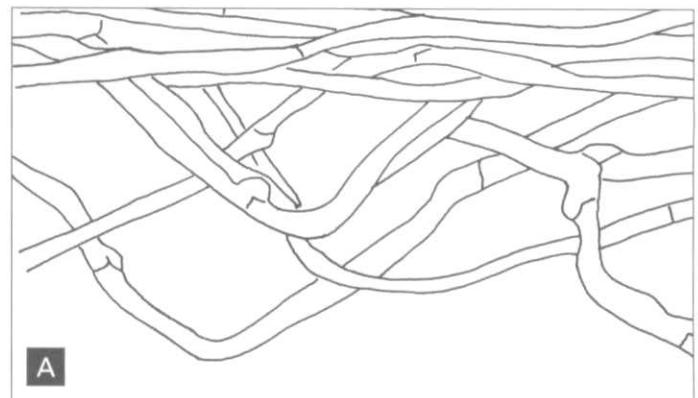


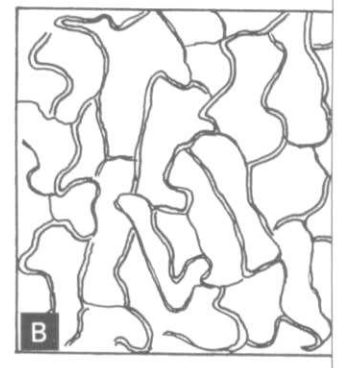





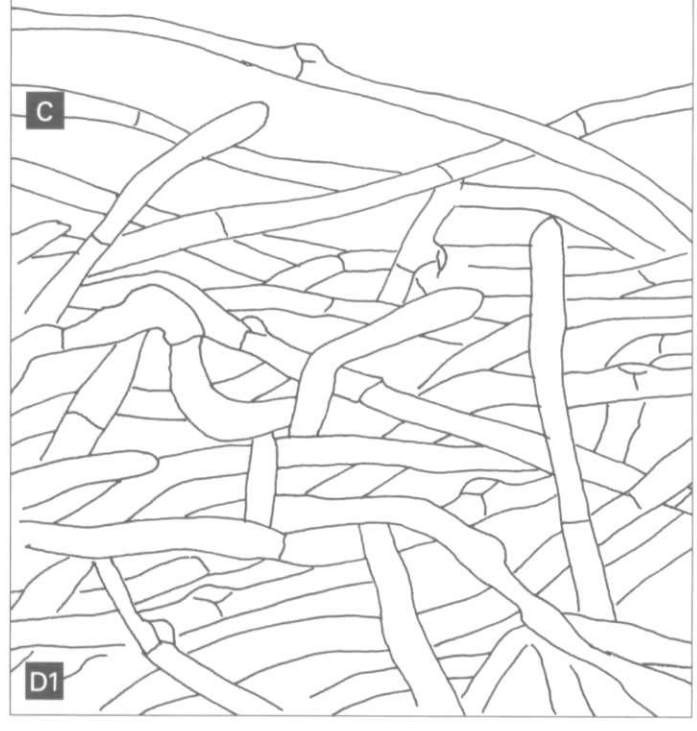


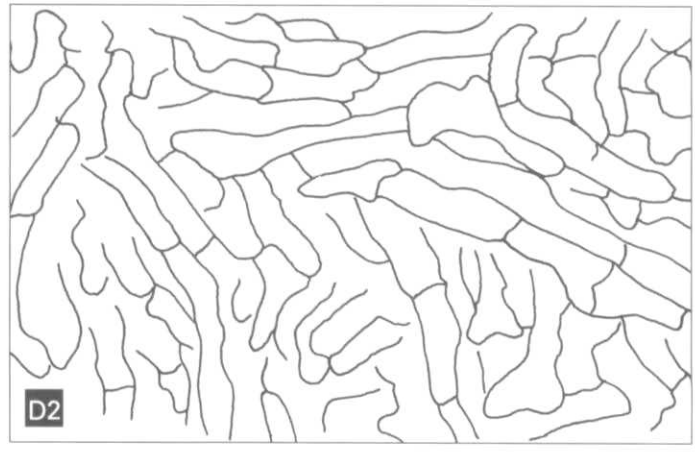






20 µm





Paxillus involutus

(Batsch) Fr.

Identification:
Synthesis, fruitbody links, literature descriptions

Macroscopic appearance

Mycorrhizas are very long and tortuous, with numerous irregularly spaced branches typically occurring along one main axis. The main axis is <16 mm in length and <0.6 mm in diameter.

Globose sclerotia (<0.8 mm in diameter) are frequently found loosely connected to the mycorrhiza by means of the dense wefts of hyphae or abundant simple strands (<0.1 mm in diameter). Young mycorrhizas are typically covered by a dense fringe of emanating hyphae.

Mycorrhizas, strands, sclerotia and hyphae are all concolorous. They are silver- white when young, changing to silver-buff (52) and then silver-bay (19) with age, and are easily bruised to produce darker patches.

Microscopic appearance

A. *Strands:* differentiated, but loosely organised, composed of intertwining hyphae 2.5–5.0 µm in diameter, with occasional clamp-connections.
Associated hyphae: of variable diameter (2.5–5.0 µm), with abundant clamp-connections.
B. *Sclerotia:* the surface rind is a net synenchyma of distinctly elongate cells, 15–25 µm in length and 4–10 µm in diameter, with thickened cell walls.
C. *Mantle edge:* loosely formed.
Emanating hyphae: like those associated with the strands and extending up to 160 µm from the mantle surface.
Specialised elements: not observed.
D. *Mantle:* 15–30 µm in depth.
D1. *Surface:* a net prosenchyma of loosely interwoven hyphae, 4–7 µm in diameter, with frequent clamp-connections.
D2. *Inner:* a net synenchyma of interwoven, elongate cells, <15 µm in length and <6 µm in diameter.

Distinguishing features

This mycorrhiza can be identified by its macroscopic features alone when fresh material is examined. Mycorrhizas of *Leccinum* spp. and other boletes may also be distinguished by their compact strands, sclerotial rinds and hyphae lacking clamp-connections.

Ecology

Mycorrhizas have been found on coniferous and broadleaved trees of all ages growing in a wide range of habitats. We have found it particularly dominant on coal waste sites in Scotland.

Fruitbody observations suggest that it is most commonly associated with *Betula* spp.

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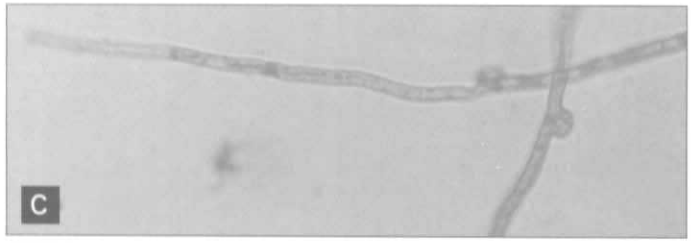
Associated trees:
Betula pendula
Betula pubescens

Class: **BASIDIOMYCOTINA**
Order: **AGARICALES**
Family: **CORTINARIACEAE**

19

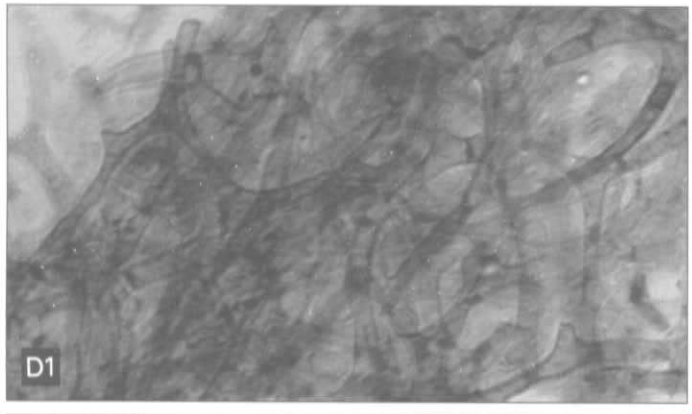
Inocybe lacera

(Fr.) Quél.


C


C


C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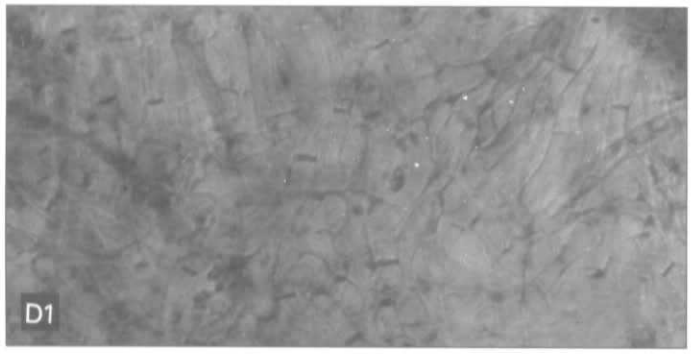
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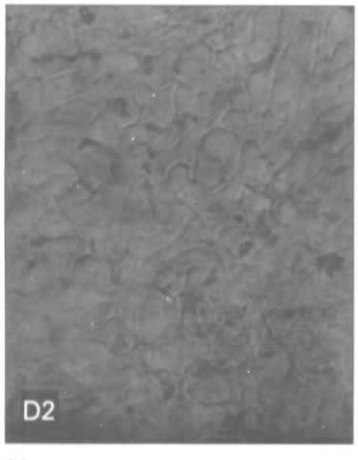
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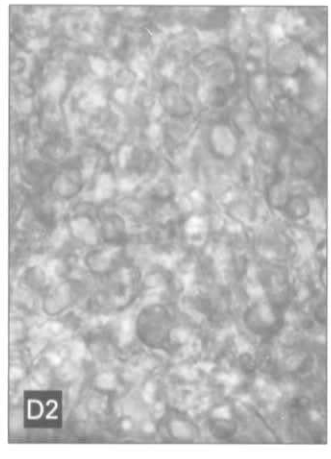
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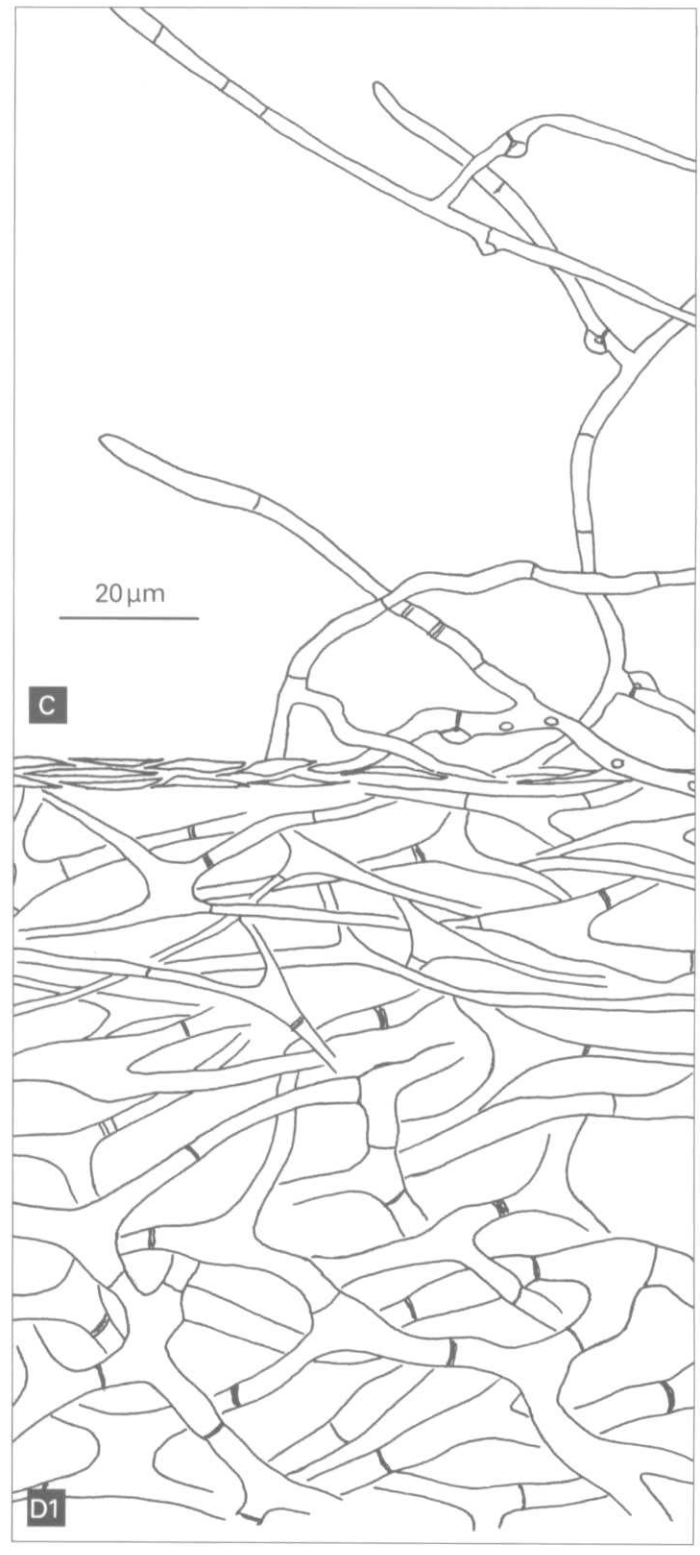
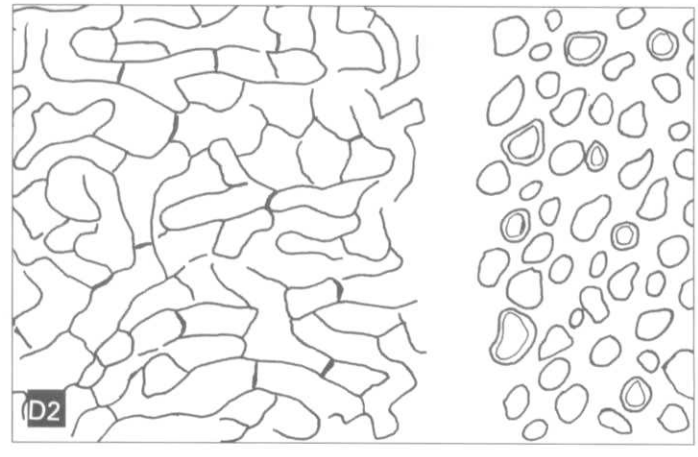

20 µm
C
D1


D2

Inocybe lacera

(Fr.) Quél.

Identification:
Synthesis, fruitbody links

Macroscopic appearance

Mycorrhizas are short with a frequent short branching habit. The main axis is <5 mm in length and <0.4 mm in diameter.

Loose hyphae are occasionally found emanating from the mantle surface, which is conspicuously smooth and shiny.

Mycorrhizas are white when young, slowly browning with age.

Microscopic appearance

A. *Strands:* not observed.
B. *Sclerotia:* not observed.
C. *Mantle edge:* smooth but not compact, consisting of distinctly adpressed hyphae.
   Emanating hyphae: narrow (1.5–2.5 µm in diameter) with frequent and often distinctly bulbous clamp-connections. Although tightly formed, these clamp-connections often possess a distinctive 'hole' — a space formed between the arching clamp and the parent hypha. Hyphae found close to the mantle surface have thick-walled septa which stain strongly in lactophenol cotton blue or toluidine blue.
   Specialised elements: not observed.
D. *Mantle:* 10–20 µm in depth.
   D1. *Surface:* a net prosenchyma of distinctly arranged, shortened, dichotomously branched hyphae, 1.5–5.0 µm in diameter. These hyphae also possess distinct septa which stain strongly in lactophenol cotton blue or toluidine blue, giving the surface a characteristic appearance.
   As the mantle ages, these hyphal cells become shorter and broader and interlock more closely.
   D2. *Inner:* a net synenchyma of shortened, interlocked cells, <15 µm in length and <8 µm in diameter. These cells are frequently characterised by large numbers of oily globules, which make the lower mantle features difficult to examine.

Distinguishing features

Like other *Inocybe* spp. examined, it can be distinguished macroscopically by the short, stubby, shiny, white mycorrhizas, and microscopically by the distinctive appearance of the mantle surface.

Separation at species level is difficult, and we have not so far been able to distinguish this mycorrhiza from those formed by *Inocybe lanuginella* (Schroet.) Konrad & Maublanc. However, mycorrhizas of *Inocybe petiginosa* (Fr.: Fr.) Gillet can be distinguished by the banding of the surface hyphae and the lack of distinctly 'open' clamp-connections with strongly staining septa.

Ecology

Mycorrhizas have frequently been found in association with *Betula* spp. on coal waste sites in Midlothian, Scotland. Both *Inocybe lacera* and *I. lanuginella* readily colonise tree seedlings and can be considered 'early stage' fungi.

Fruitbody observations suggest that *I. lacera* is also commonly associated with *Pinus* spp.

References

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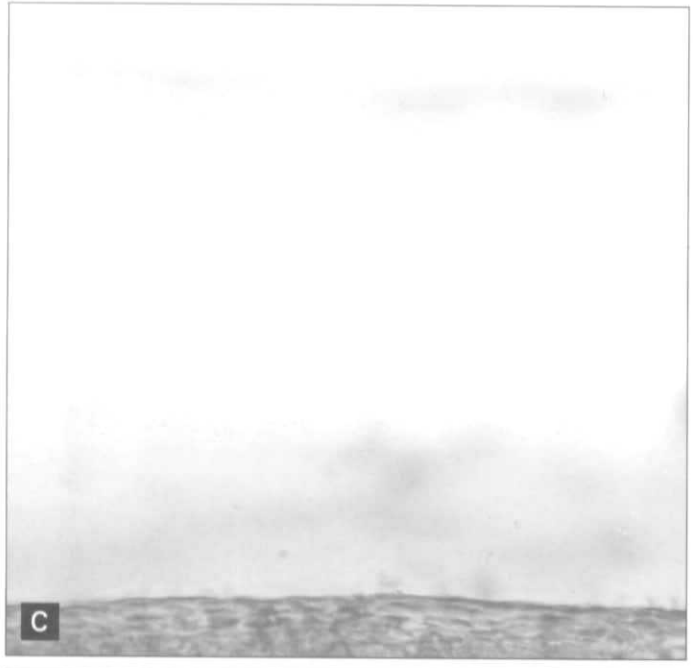
Associated trees:
Betula pendula
Betula pubescens

Class: **BASIDIOMYCOTINA**
Order: **RUSSULALES**
Family: **RUSSULACEAE**

20

Lactarius glyciosmus

(Fr.: Fr.) Fr.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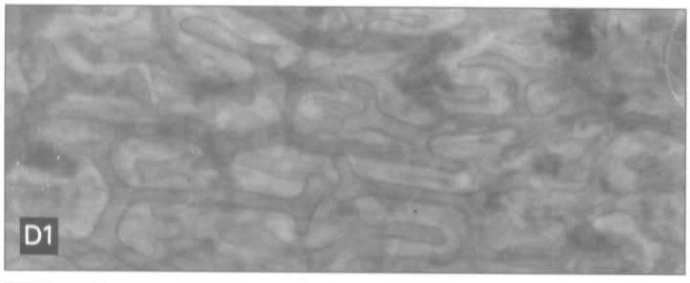
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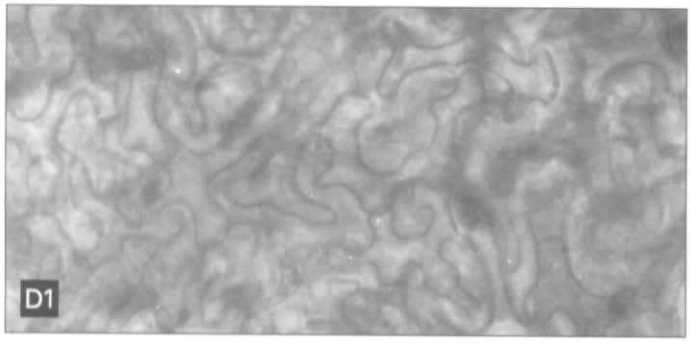
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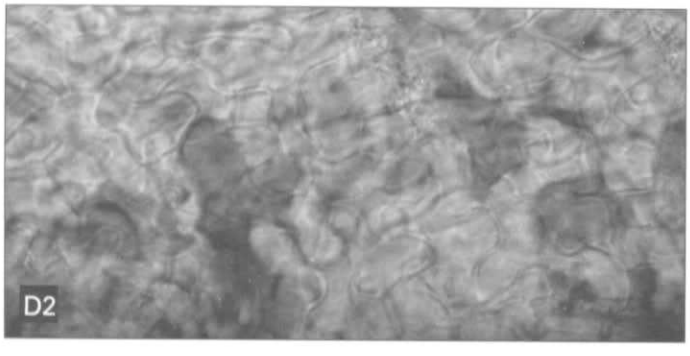
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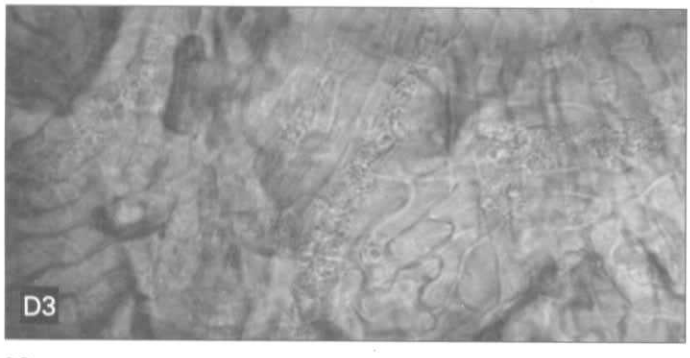
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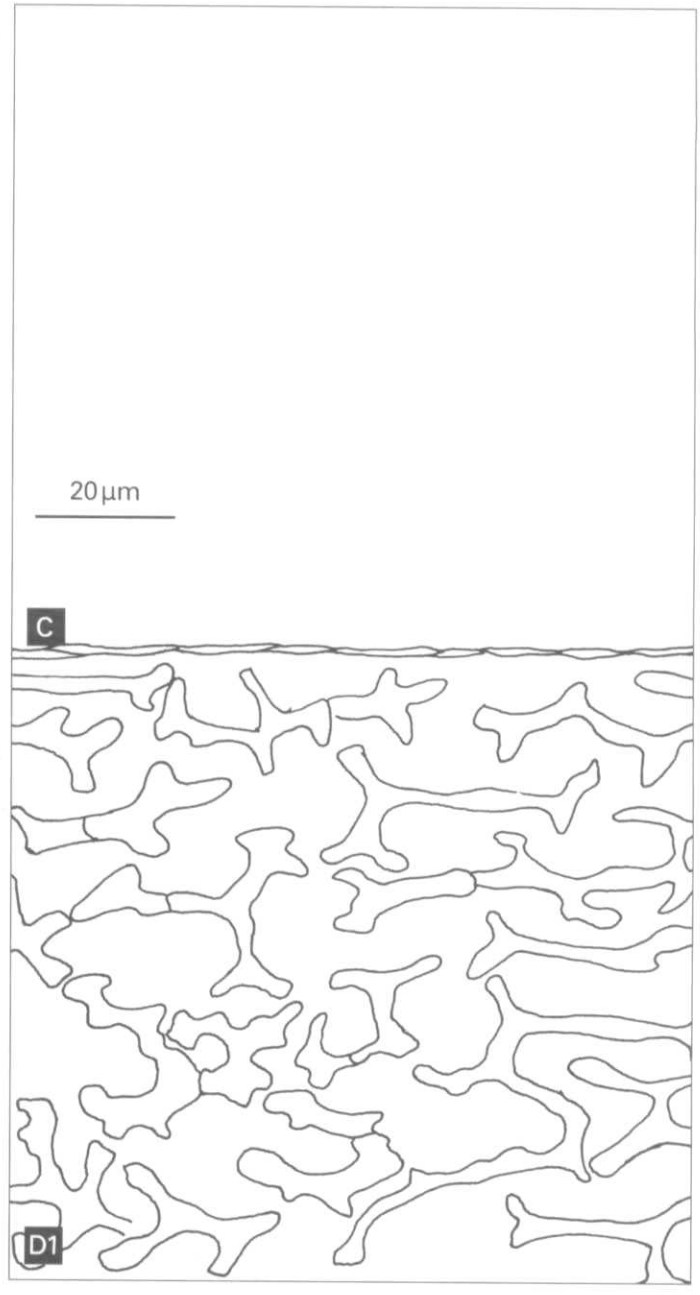
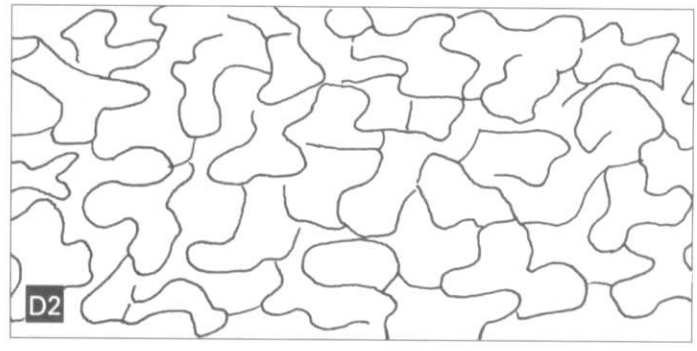

20 µm
C
D1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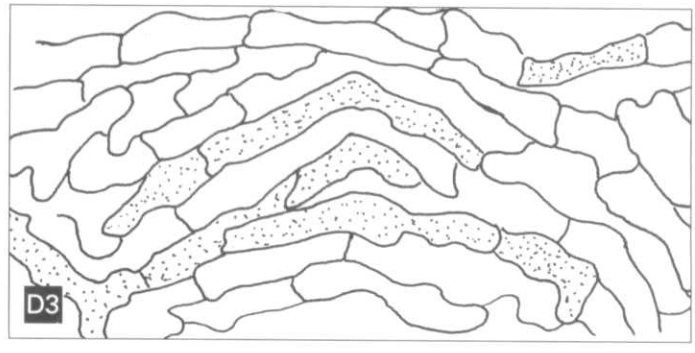
D3

Lactarius glyciosmus

(Fr.: Fr.) Fr.

Identification:
Fruitbody links, literature descriptions, fruitbody taxonomy

Macroscopic appearance

Mycorrhizas are fairly short with a frequent, sometimes pinnate, branching pattern. The main axis is <6 mm in length and <0.4 mm in diameter.

The mantle surface is smooth and shiny, and loose hyphae and strands are not observed.

Mycorrhizas are white to smoke-grey (34) when young, changing to different shades of brown with age.

Microscopic appearance

A. *Strands:* not observed.
B. *Sclerotia:* not observed.
C. *Mantle edge:* Smooth and compact in appearance.
Emanating hyphae and specialised elements: not observed.
D. *Mantle:* 15–25 µm in depth.
D1. *Surface:* a net synenchyma of distinctly shaped hyphal cells which stain strongly pink in toluidine blue. In the young mycorrhiza, these cells are elongated and thin (2–4 µm in diameter) but, as the mycorrhiza ages, the cells become shorter and broader (3–5 µm in diameter).
D2. *Intermediate:* an irregular interlocking synenchyma of cells 3–10 µm in diameter.
D3. Inner: a net synenchyma of septate hyphae 2–5 µm in diameter. Running through this layer are larger, granular, laticiferous hyphae, 3–6 µm in diameter.

All mantle features are best seen when stained in toluidine blue.

Distinguishing features

This mycorrhiza is characteristic of several *Lactarius* spp., with its smooth, compact, multi-layered mantle containing laticiferous hyphae and staining best in toluidine blue. It is distinguished from those of *Lactarius rufus* (Scop.: Fr.) Fr. and *Lactarius pubescens* (Fr.: Krombh.) Fr. by its distinctive mantle surface, which stains pink in toluidine blue, and by the infrequent formation of strands.

Ecology

Mycorrhizas have been widely recorded on 5–15 year old *Betula* spp. growing on brown earth sites near Edinburgh. These mycorrhizal populations have invariably produced large numbers of *L. glyciosmus* fruitbodies.

Fruitbody observations suggest that this fungus may be specific to *Betula* spp.

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FLEMING, L.V. 1983. *Establishment, persistence and spread of sheathing mycorrhizal fungi on roots of birch* (Betula *spp.*). PhD thesis, University of Edinburgh.

GODBOUT, C. & FORTIN, J.A. 1985. Synthesized ectomycorrhizae of aspen: fungal genus level of characterization. *Can. J. Bot.*, **63,** 252–262.

MÜNZENBERGER, B., METZLER, B., KOTTKE, I. & OBERWINKLER, F. 1986. Morphological and anatomical characterization of the mycorrhiza *Lactarius deterrimus — Picea abies in vitro. Z. Mykol.*, **52,** 407–422.

VOIRY, H. 1981. Classification morphologique des ectomycorrhizes du chêne et du hêtre dans le nord-est de la France. *Eur. J. For. Path.*, **11,** 284–299.

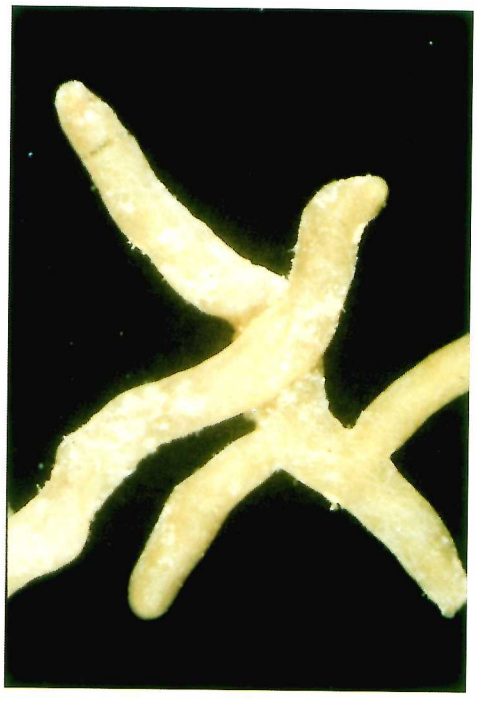
Associated trees:
Betula pendula
Betula pubescens

Class: **BASIDIOMYCOTINA**
Order: **RUSSULALES**
Family: **RUSSULACEAE**

21

Lactarius pubescens

(Fr.: Krombh.) Fr.

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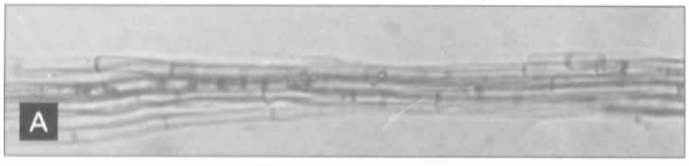
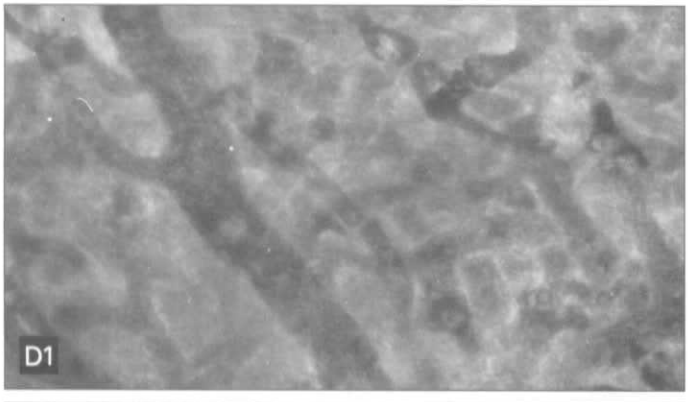

A


C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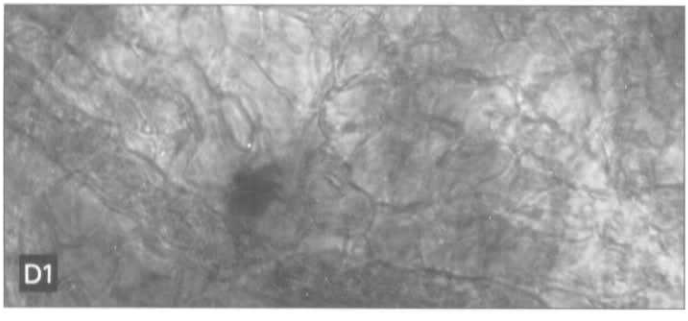
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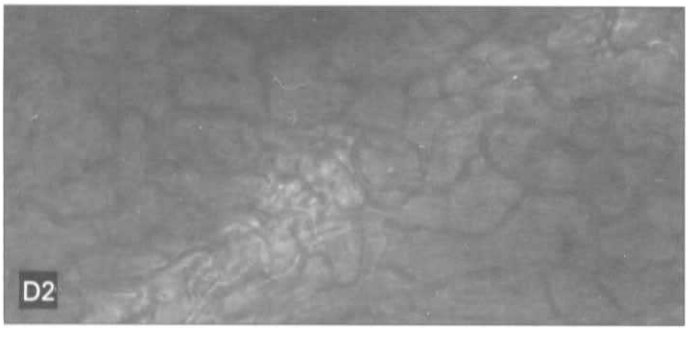
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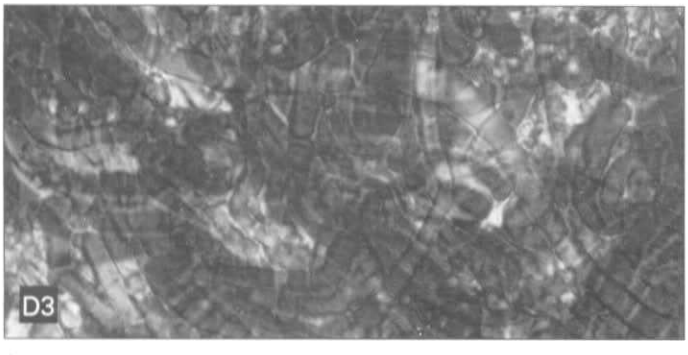
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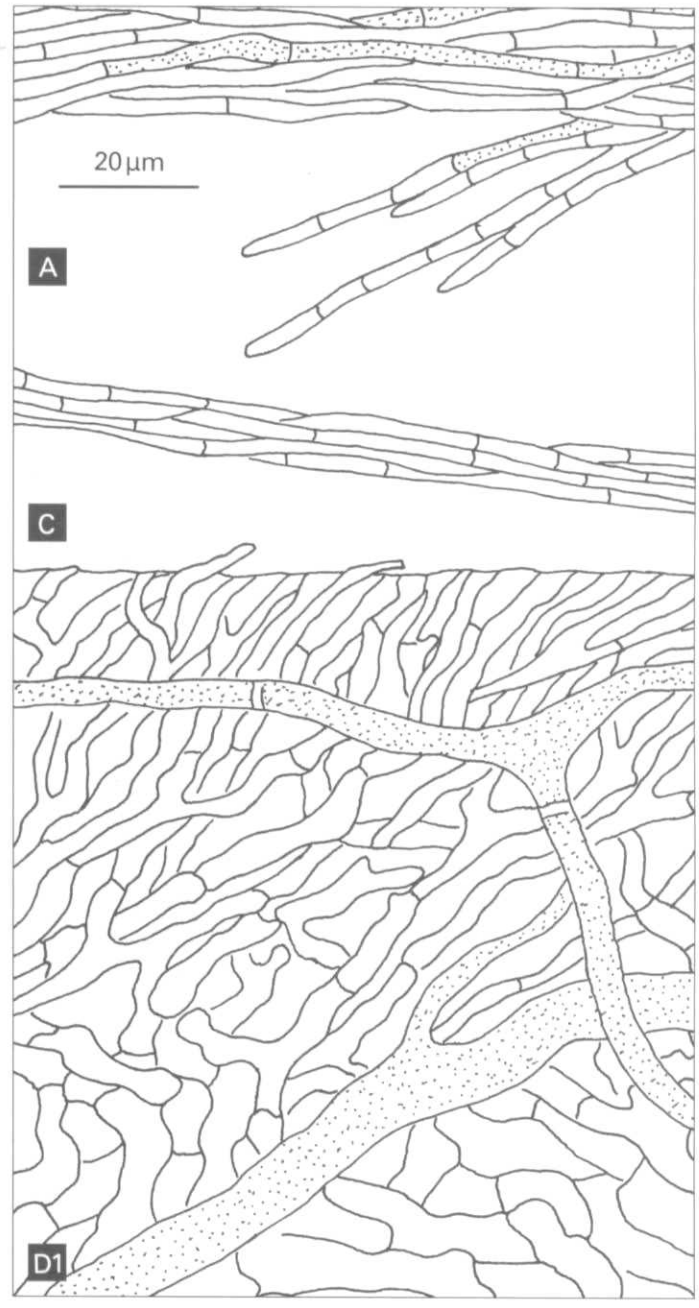
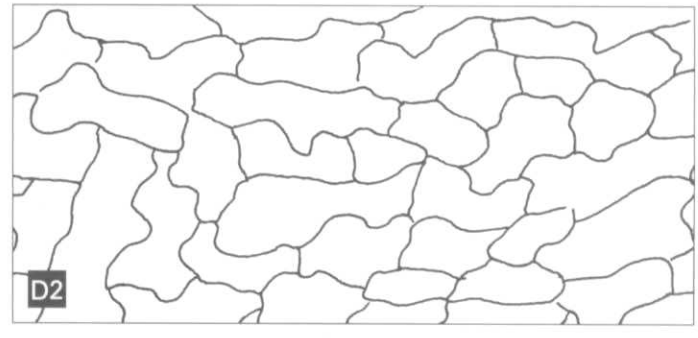

20 µm
A
C
D1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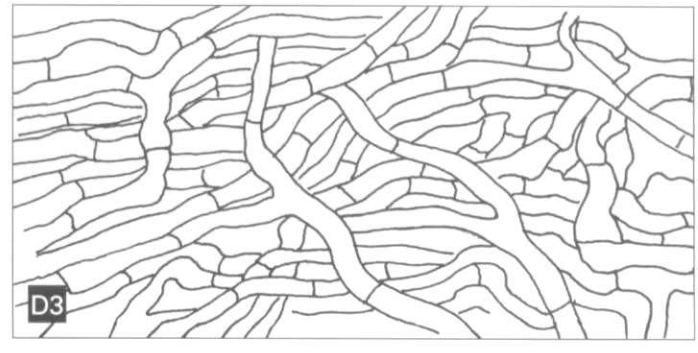
D3

Lactarius pubescens

(Fr.: Krombh.) Fr.

Identification:
Synthesis, fruitbody links, literature descriptions, fruitbody taxonomy

Macroscopic appearance

Mycorrhizas are fairly long, with a frequent, sometimes pinnate, branching pattern. The main axis is <10 mm in length and <0.4 mm in diameter.

The mantle surface is smooth and shiny, covered by a network of paler hyphae which are just visible under the dissecting microscope. Mycelial strands are invariably present, being smooth and compact, up to 0.2 mm in diameter, and frequently branched. They are often found arising from the base of the mycorrhiza.

Mycorrhizas and strands are concolorous, being cream (4) when young, changing to apricot (47) with age.

Microscopic appearance

A. *Strands:* a compact, largely undifferentiated, structure of parallel hyphae, which are thin (<3 µm in diameter), straight, septate and lacking clamp-connections. In older strands, a second type of granular, laticiferous hyphae (<6 µm in diameter) is often found running through the centre of the strand.
   Associated hyphae: occasionally found, thin (2–3 µm in diameter), strongly septate, with cells usually <25 µm in length. Clamp-connections are rarely seen.
B. *Sclerotia:* not observed.
C. *Mantle edge:* smooth and compact in appearance.
   Emanating hyphae and specialised elements: not observed.
D. *Mantle:* 10–25 µm in depth.
   D1. *Surface:* a net synenchyma of hyphae, 2–5 µm in diameter, covered by a widely spaced network of granular, laticiferous hyphae up to 8 µm in diameter, septate, with frequent dichotomous branching.
   As the mantle ages, the hyphae are broader (<10 µm in diameter), forming an irregular non-interlocking synenchyma still overlain by a network of laticiferous hyphae.
   D2. *Intermediate:* an irregular interlocking synenchyma of cells, <15 µm in length and <10 µm in diameter.
   D3. *Inner:* a net synenchyma of dichotomously branched hyphae 2–5 µm in diameter.

   All mantle features are best seen when stained in toluidine blue.

Distinguishing features

This mycorrhiza is characteristic of several *Lactarius* spp. in possessing a smooth, compact, multi-layered mantle containing laticiferous hyphae and staining best in toluidine blue. It is distinguished from those of *Lactarius glyciosmus* (Fr.: Fr.) Fr. and *Lactarius rufus* (Scop.: Fr.) Fr. by its abundant strand-forming habit and the presence of a distinctive network of laticiferous hyphae covering the mantle surface.

Ecology

Mycorrhizas have been widely recorded on 3–10 year old *Betula* spp. growing on brown earth sites near Edinburgh.

Fruitbody observations suggest that this fungus may be specific to *Betula* spp.

References

BRAND, F. & AGERER, R. 1986. Studies on ectomycorrhizae VIII. *Z. Mykol.*, **52,** 287–320.

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MÜNZENBERGER, B., METZLER, B., KOTTKE, I. & OBERWINKLER, F. 1986. Morphological and anatomical characterization of the mycorrhiza *Lactarius deterrimus — Picea abies in vitro. Z. Mykol.*, **52,** 407–422.

VOIRY, H. 1981. Classification morphologique des ectomycorrhizes du chêne et du hêtre dans le nord-est de la France. *Eur. J. For. Path.*, **11,** 284–299.

Associated trees:
Betula pendula
Betula pubescens
Picea sitchensis
Pinus sylvestris

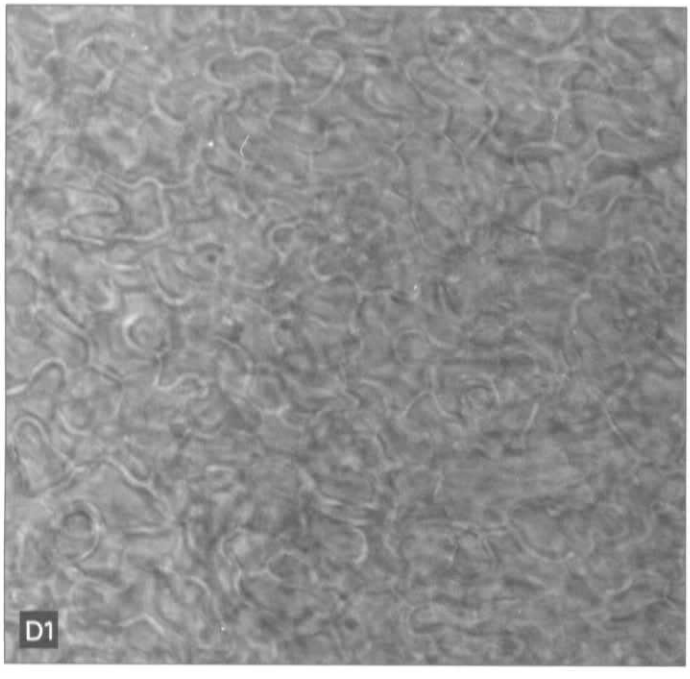
Class: **BASIDIOMYCOTINA**
Order: **RUSSULALES**
Family: **RUSSULACEAE**

22

Lactarius rufus

(Scop.: Fr.) Fr.


C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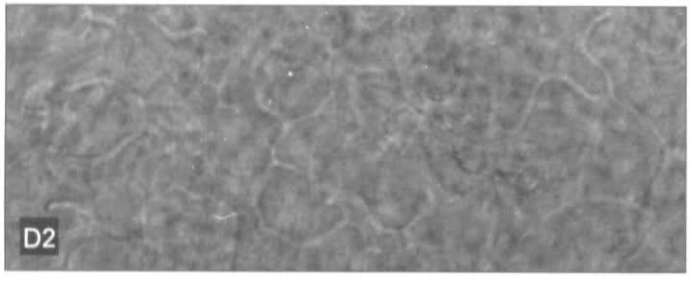
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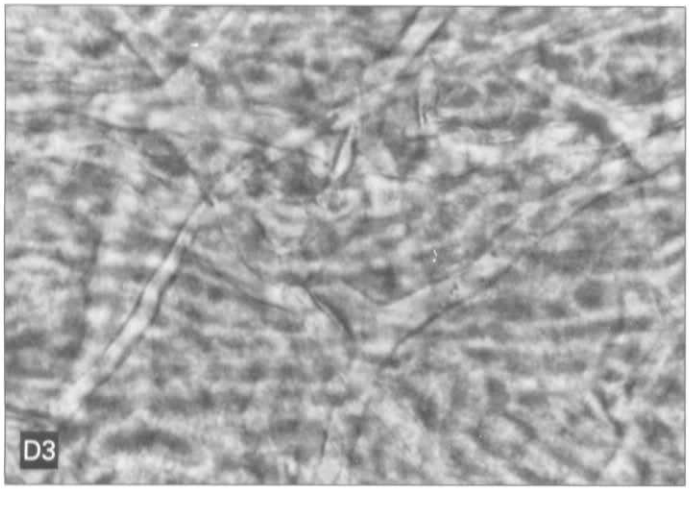
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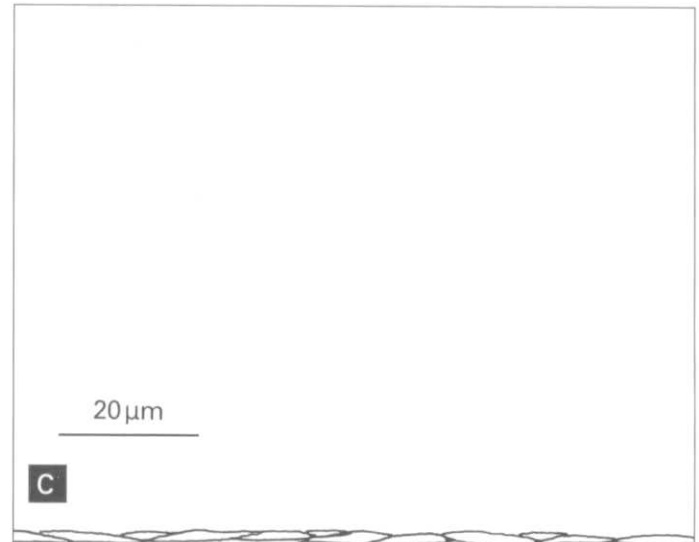
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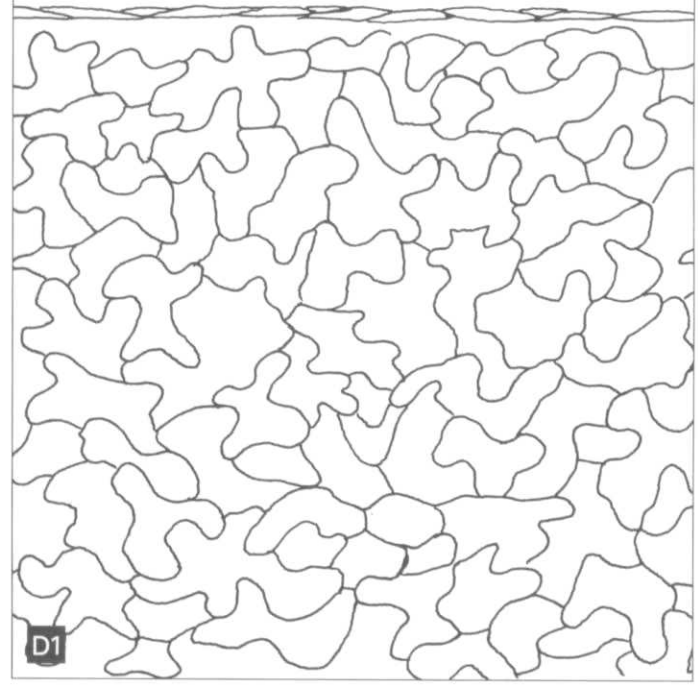
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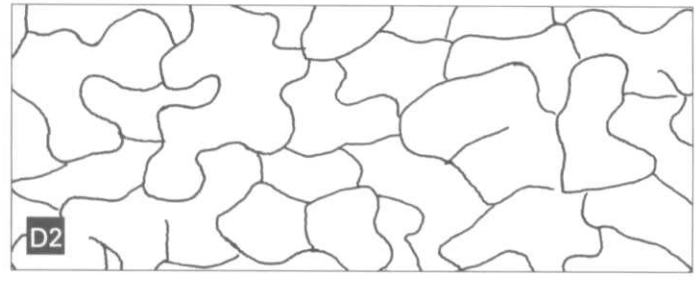
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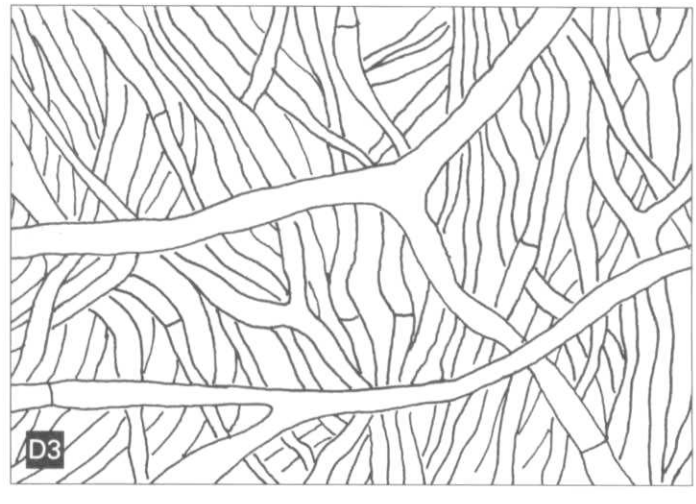
D3

Lactarius rufus

(Scop.: Fr.) Fr.

Identification:
Synthesis, fruitbody links, literature descriptions, fruitbody taxonomy

Macroscopic appearance

Mycorrhizas are long and fairly straight, exhibiting a frequent, sometimes pinnate, branching pattern. The main axis is <12 mm in length and <0.5 mm in diameter.

The mantle surface is smooth and shiny, and loose hyphae and strands are rarely observed.

Mycorrhizas are pale buff (52) when young, changing to dark sienna-brown (11) with age.

Microscopic appearance

A. *Strands:* rarely observed.
B. *Sclerotia:* not observed.
C. *Mantle edge:* Smooth and compact in appearance.
Emanating hyphae: rarely observed but, when present, 3–4 µm in diameter, septate and lacking clamp-connections.
Specialised elements: not observed.
D. *Mantle:* 15–25 µm in depth.
D1. *Surface:* an irregular interlocking synenchyma of cells, 3–15 µm in diameter, which produce a distinctive 'jigsaw'-like appearance. Very little change in structure is observed with age.
D2. *Intermediate:* an irregular non-interlocking synenchyma of cells, 7–15 µm in diameter.
D3. *Inner:* a net synenchyma composed mainly of septate hyphae 2–4 µm in diameter. Running through this layer are larger laticiferous hyphae (3–8 µm in diameter), septate, with frequent dichotomous branching.

All mantle features are best seen when stained in toluidine blue.

Distinguishing features

This mycorrhiza is characteristic of several *Lactarius* spp. in possessing a smooth, compact, multi-layered mantle containing laticiferous hyphae and staining best in toluidine blue. It is distinguished from those of *Lactarius glyciosmus* (Fr.: Fr.) Fr. and *Lactarius pubescens* (Fr.: Krombh.) Fr. by its distinctive 'jigsaw'-like mantle surface and infrequent formation of strands.

Ecology

Mycorrhizas occur on a wide range of trees, particularly conifers, of over 5 years of age.

Fruitbody observations suggest that it is also a common associate of *Betula* spp., particularly on coal waste sites in Scotland.

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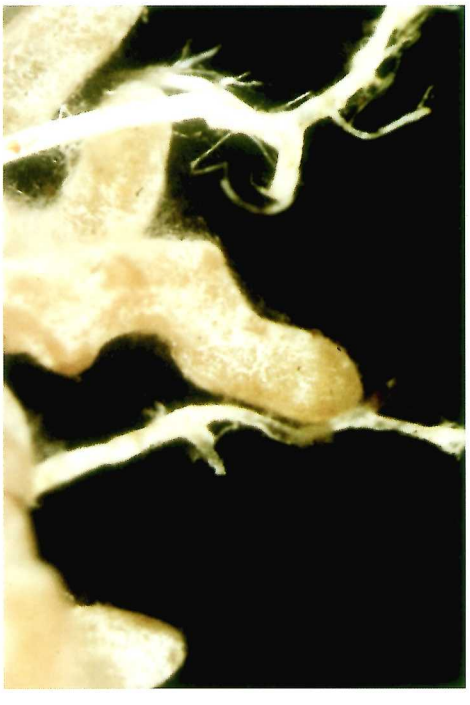
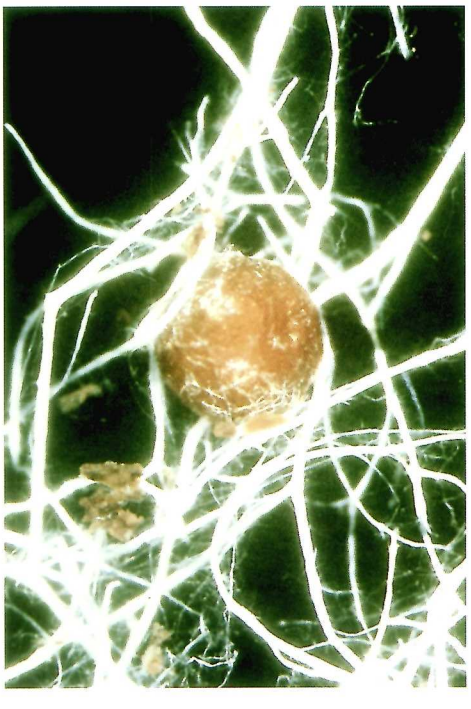
Associated trees:
Betula pendula
Betula pubescens
Quercus robur

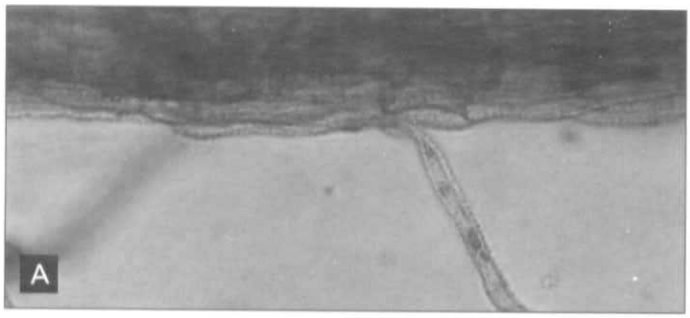
Class: **BASIDIOMYCOTINA**
Order: **BOLETALES**
Family: **BOLETACEAE**

23

Leccinum sp.



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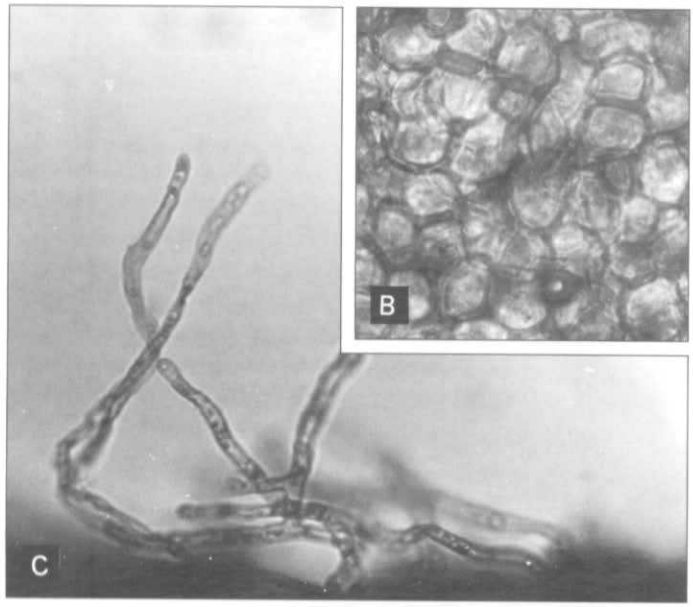
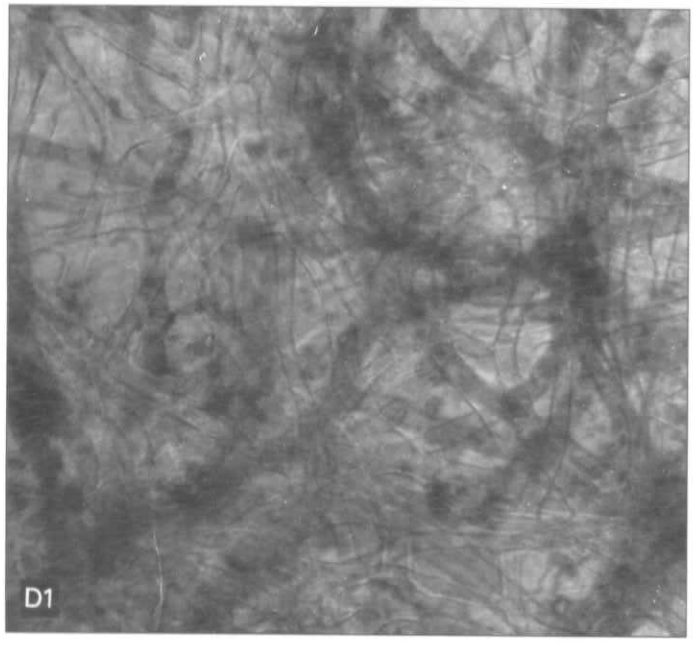

B
C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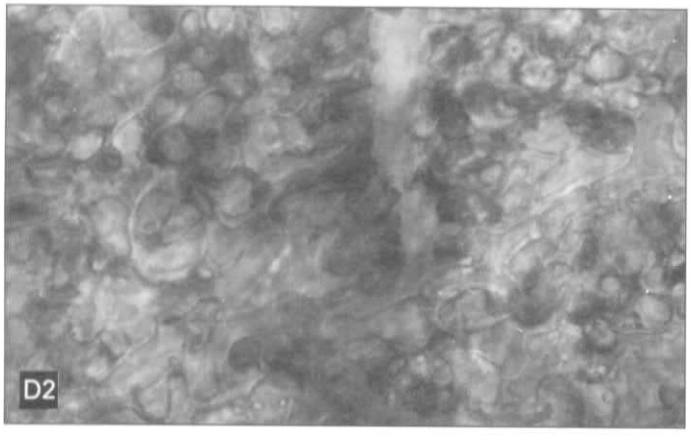
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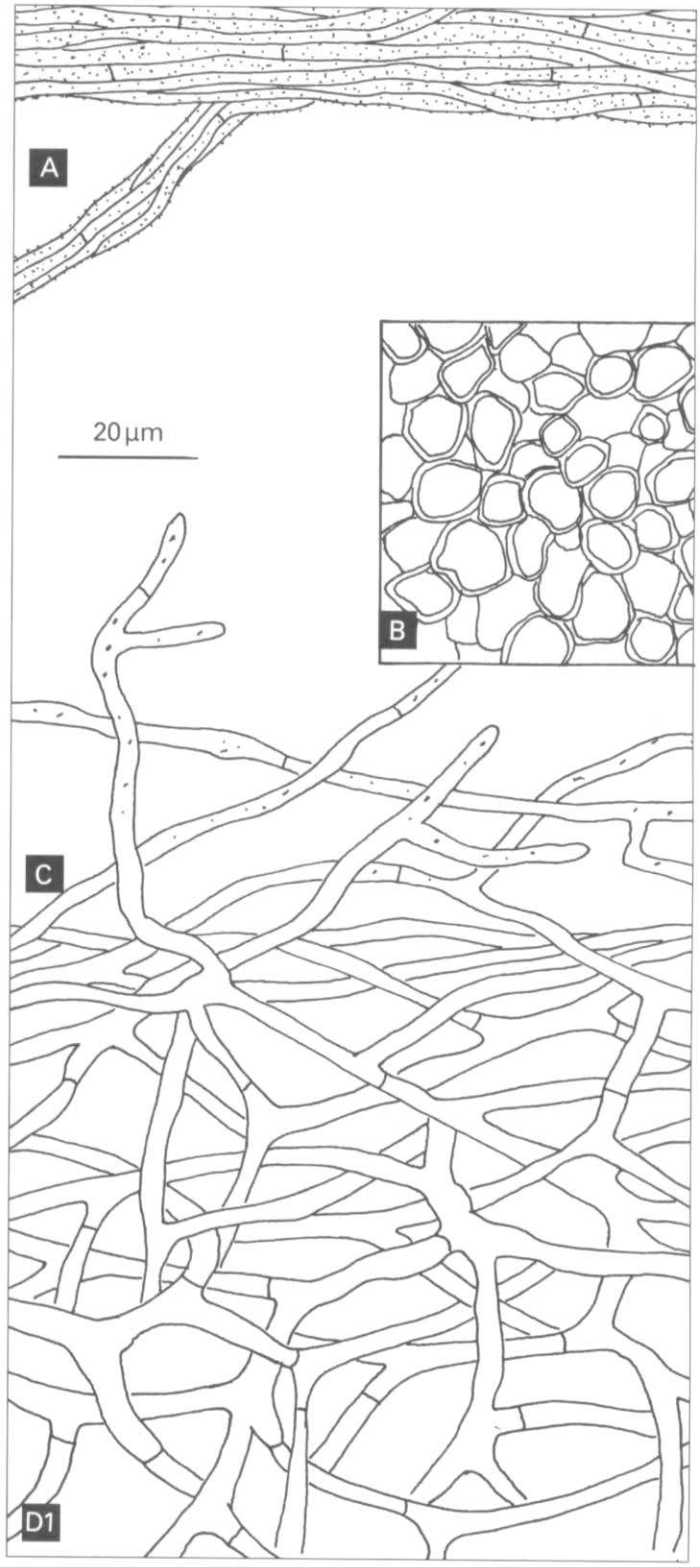
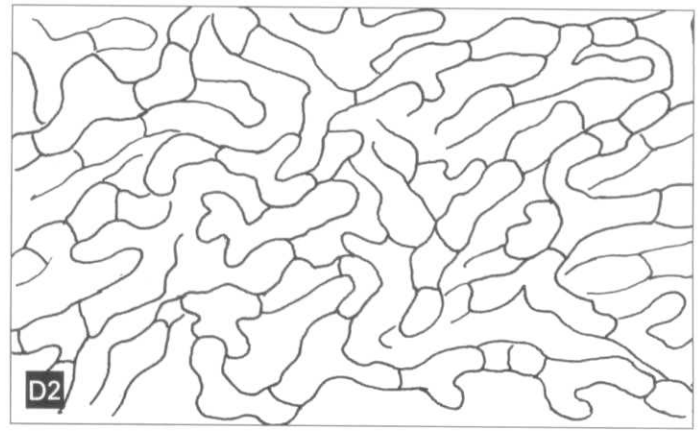

A
20 µm
B
C
D1


D2

Leccinum sp.

Identification:
Literature descriptions, fruitbody taxonomy

Macroscopic appearance

Mycorrhizas are tortuous, with frequent, irregular-spaced branching, often forming dense clusters. The main axis is <7 mm long and <0.5 mm in diameter.

The mantle surface is covered by dense wefts of hyphae when young which disappear with age. Mycelial strands are invariably present, and are of large diameter (<0.6 mm), highly branched, smooth and compact. Like the mantle, they are covered by a dense fringe of hyphae when young. Sclerotia, although rarely found, have been recorded firmly attached to these strands.

Mycorrhizas are silver-white when young, changing to silver-saffron (49) to fulvous (12) with age or on bruising. Strands are white, yellowing slowly with age. Sclerotia, where recorded, have been rusty-tawny (14).

Microscopic appearance

A. *Strands:* compact and differentiated. The surface and associated hyphae are <3 µm in diameter, septate, lacking clamp-connections, and are usually distinctly verrucose. Most of these strands are differentiated with a central core of wider hyphae <6 µm in diameter.
B. *Sclerotia:* the surface rind consists of thick-walled, irregularly shaped cells (5-sided to globose), 6–7 µm in diameter, forming an irregular non-interlocking synenchyma.
C. *Mantle edge:* loosely formed.
   Emanating hyphae: extending up to 80 µm from the mantle surface, <3 µm in diameter, septate, lacking clamp-connections, and usually not verrucose. Branching is frequently found, but often only a single branch occurs on each extending hypha. These hyphae may be absent in old mycorrhizas.
   Specialised elements: not observed.
D. *Mantle:* 10–15 µm in depth.
   D1. *Surface:* a loosely organised network of hyphae, 3–6 µm in diameter, forming a net prosenchyma. These hyphae are often dichotomously branched, producing a characteristic appearance.
   D2. *Inner:* a net synenchyma of tortuous cells, 5–20 µm in length and 3–6 µm in diameter. These cells are often distinctly bulbous, with narrowing at the septa.

Distinguishing features

This mycorrhiza is distinguished from those formed by *Paxillus involutus* (Batsch.: Fr.) Fr. and *Scleroderma citrinum* Pers. by macroscopic features of large, compact, branched strands and microscopic features of hyphae lacking clamp-connections. Although very similar, mycorrhizas of *Suillus* spp. appear to be distinguished by the presence of abundant glutinous deposits on the hyphae and mantle surface. We have so far been unable to distinguish between different species of *Leccinum*.

Ecology

Mycorrhizas have commonly been observed on *Betula* spp. of 5–15 years of age growing on brown earth and coal waste sites in Midlothian, Scotland. We have also observed similar mycorrhizas in a mature birch wood in Perthshire, Scotland, and in association with 3–9 year old *Quercus robur* L. growing in a SE England nursery.

Fruitbody observations suggest that *Leccinum* spp. have a wide host range, but are predominantly associated with *Betula* spp.

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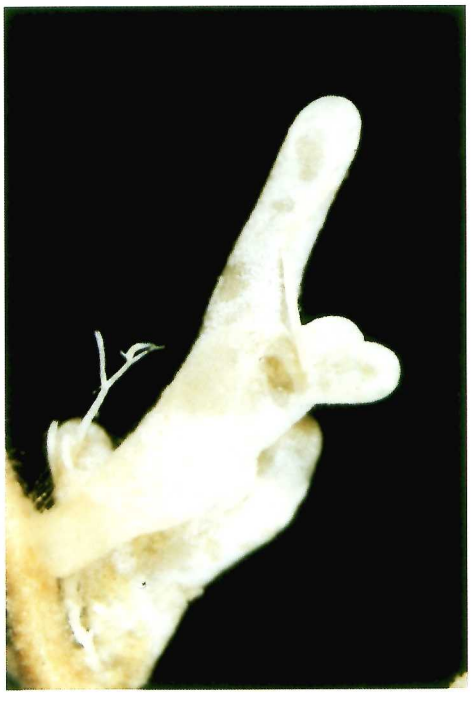
Associated trees:
Betula pendula
Picea abies
Pinus sylvestris

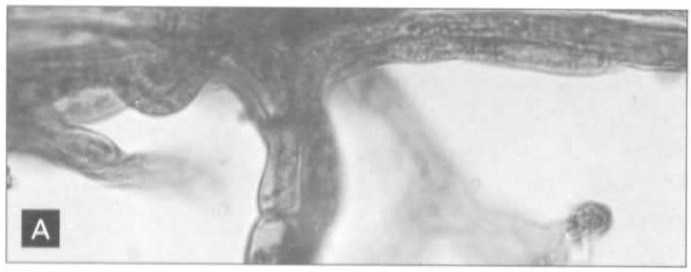
Class: **BASIDIOMYCOTINA**
Order: **AGARICALES**
Family: **AMANITACEAE**

24

Amanita muscaria

(L.: Fr.) Hooker

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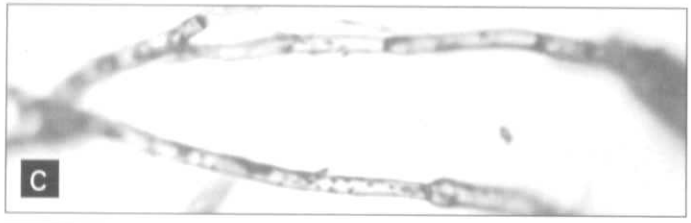
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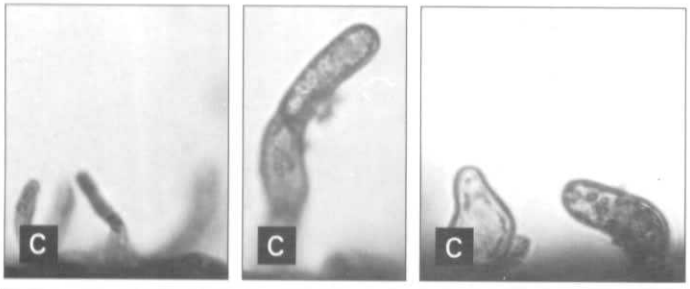
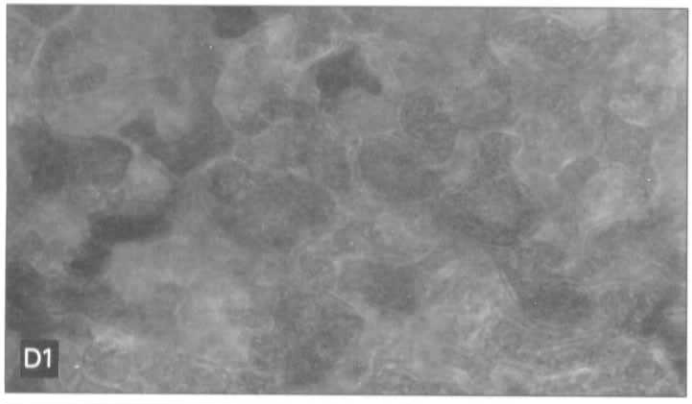
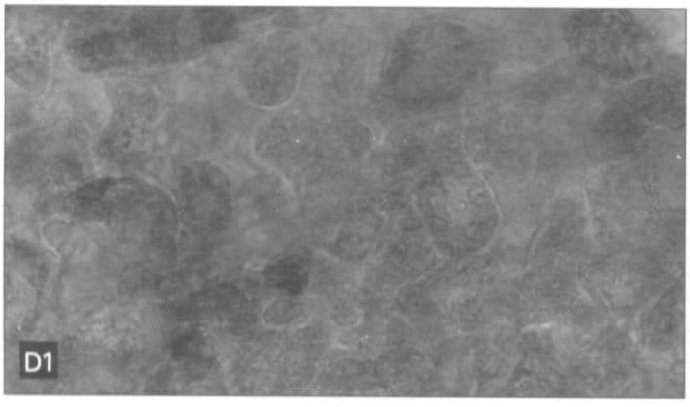

C
C
C


D1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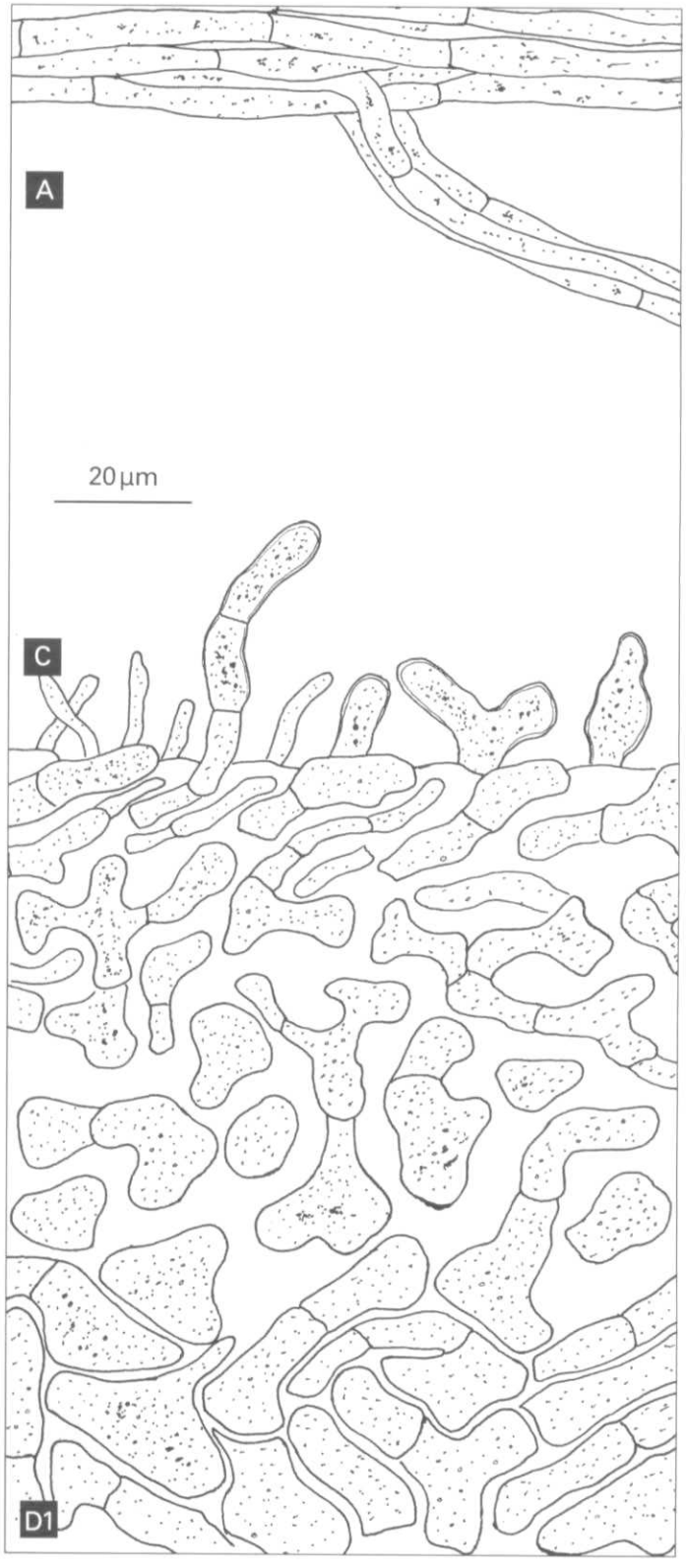
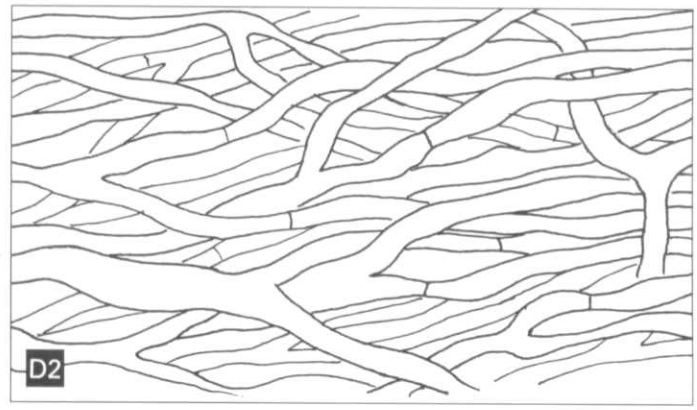
D2


A
20 µm
C
D1


D2

Amanita muscaria

(L.: Fr.) Hooker

Identification:
Synthesis, fruitbody links

Macroscopic appearance

Mycorrhizas are short, stubby and tortuous, with frequent irregularly spaced branches often forming quite dense clusters. The main axis is <5 mm in length and <0.6 mm in diameter.

The mantle surface is roughened and distinctly granular in appearance. Loose hyphae are found only occasionally, but smooth, frequently branched strands <0.1 mm in diameter are often present.

Mycorrhizas are silver-white when young, changing to fawn (29) with age or on bruising. Strands are white, also turning fawn.

Microscopic appearance

A. *Strands:* smooth, compact and undifferentiated. Hyphae are 3–8 µm in diameter, lacking clamp-connections and often composed of inflated cells narrowing at the septa. These cells also frequently contain noticeably granular cytoplasm.
B. *Sclerotia:* not observed.
C. *Mantle edge:* compact and very uneven.
Emanating hyphae: rarely present, but septate hyphae, <3 µm in diameter and lacking clamp-connections, have been observed.
Specialised elements: short, cystidium-like, septate hyphae, with granular cytoplasm extending up to 20 µm from the mantle surface. These hyphae may be <3 µm in diameter, narrowing at the tip. However, a second form is distinctly swollen and bulbous (<15 µm in diameter), often branched, forming a wide variety of shapes. All of these cystidial elements occur most frequently on young mycorrhizas.
D. *Mantle:* 25–30 µm in depth.
D1. *Surface:* a net prosenchyma of hyphal or bulbous elements formed by the cystidium-like hyphae described in C being squashed into a plan view of the mantle surface.
In older mycorrhizas, these elements appear broader (5–25 µm in diameter) and become compacted to form a non-interlocking irregular synenchyma.
D2. *Inner:* a net synenchyma of septate, branched hyphae 3–8 µm in diameter.

Distinguishing features

Several mycorrhizal workers have described *Amanita* mycorrhizas, particularly those of *A. muscaria*, as possessing a 'hoarfrost' surface. Microscopic examination of the mantle surface shows that this appearance is due to the presence of distinctive cystidium-like hyphae. These features appear to distinguish *Amanita* mycorrhizas from those formed by members of the *Boletaceae*. It is possible that the particular features of these cystidium-like hyphae may specifically distinguish *A. muscaria* but, as yet, mycorrhizas formed by other species of *Amanita* have not been examined microscopically.

Ecology

Mycorrhizas have been found on mature *Betula* and *Pinus* spp. growing in a wide range of habitats. The failure of this fungus to infect tree seedling roots growing in unsterile soils, and our failure to observe its mycorrhizas on trees less than 15–20 years of age indicate that it is a 'late stage' fungus.

Fruitbody observations suggest that this mycorrhiza is commonest on *Betula* spp. but has a wide host range.

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Appendix I Index of fungi named in descriptions

Described fungi are shown in bold.

Name	*Number*	*Number of description where references to named fungi occur*
Amanita muscaria	24	
Amphinema byssoides	6	8
Cenococcum geophilum	15	16
Genea klotzschii		14
Hebeloma crustuliniforme		9
Hebeloma leucosarx		9
Hebeloma mesophaeum	8	6,9
Hebeloma sacchariolens	9	
Hebeloma velutipes		9
Humaria hemisphaerica	1	2,3
Inocybe lacera	19	12
Inocybe lanuginella		12,19
Inocybe petiginosa	12	19
Laccaria amethystea		10,11
Laccaria proxima	10	7,11
Laccaria tortilis	11	10
Lactarius glyciosmus	20	21,22
Lactarius pubescens	21	20,22
Lactarius rufus	22	20,21
Leccinum sp.	23	18
Mycelium radicis atrovirens		5
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Piloderma croceum		6
Scleroderma citrinum		23
Sphaerosporella brunnea		1,2
Suillus spp.		23
Thelephora terrestris	7	10,11
Tomentella sp.		7
Tricharina gilva	2	1,3
Tuber sp.	13	14
Tuber albidum		13
Tuber magnatum		13
ITE.1	3	1,2
ITE.2	4	
ITE.3	5	
ITE.4	14	13
ITE.5	16	15
ITE.6	17	

Appendix II Index of trees associated with mycorrhizas described

Full name and authority	*Number of description*
Alnus glutinosa Gaertner	18
Betula pendula Roth	4, 7–16, 18–24
Betula pubescens Ehrh.	7,10, 18–23
Quercus robur L.	13,23
Picea sitchensis (Bong.) Carr	1,12–18,22
Picea abies (L.) Karst.	24
Pinus contorta Douglas	2,7,8,10,18
Pinus sylvestris L.	7,10,15,17,18,22,24
Pseudotsuga menziesii (Mirb.) Franco	2,5,6,12,13,17,18

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Up until recently the published descriptions of ectomycorrhizas have often been insufficient for precise identification. This booklet describes a rapid but accurate method for examination and characterisation using a series of 24 descriptions of ectomycorrhizas most commonly encountered on young trees in Britain by the ITE research group. Produced for use in the laboratory, the format is designed to relate to the way in which whole root mounts are examined. It is hoped that this guide will improve the accuracy and interpretation of experimental data, facilitate communication and stimulate research, thus encouraging the identification of unknown ectomycorrhizas.

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