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STUDIES ON ECTOMYCORRHIZAE—XV*

Mycorrhizae formed by Rhizopogon luteolus on Pinus silvestris

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The mycorrhizae of *Rhizopogon luteolus* on *Pinus silvestris* are comprehensively described. They are characterized by a coralloid habit, ochre to yellow colour, highly differentiated rhizomorphs, a two-layered mantle and a differentiated autofluorescence in mantle, Hartig net and rhizomorphs. The literature on anatomical and morphological features of the mycorrhizae of *R. luteolus* and other species of *Rhizopogon* is reviewed and the features of the latter are compared with those of *R. luteolus*.

In this series ectomycorrhizae are described comprehensively, because the interpretation of physiological and ecological research on ectomycorrhizae depends on having well-known, unequivocally characterized species e.g. for studies of the application of mycorrhizae for wood production. In addition it will be essential for taxonomic and systematic reasons as data in Brand & Agerer (1986), Agerer (1987b), and Godbout & Fortin (1985) indicate common characters for genera or sections of genera.

MATERIALS AND METHODS

The method for isolation of ectomycorrhizae from soil and methods of documentation are given by Agerer (1986, 1987). Methods for examining ectomycorrhizae anatomically and morphologically and the value of features observed are discussed in detail also by Agerer (1986, 1987a).

Abbreviations.

TCt: the average of tangential dimensions of Tannin cells.

TCq: the quotient of the average of tangential dimensions divided by the average of radial dimensions of Tannin cells.

CCt: the average of tangential dimensions of cortical cells.

CCq: the quotient of the average of tangential dimensions divided by the average of radial dimensions.

* Studies on Ectomycorrhizae XIV, see Agerer (1987c).

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DESCRIPTION OF MYCORRHIZAE OF RHIZOPOGON LUTEOLUS FR.: FR. ON PINUS SILVESTRIS

Morphological characters (Fig. 1a).

Mycorrhizal system coralloid: mycorrhizae dichotomously branched: tips usually straight but sometimes bent; length of mycorrhizal system varying between 3 and 5 mm; length of unramified ends varying, up to 1 mm long and 0.35–0.4 mm in diam.; surface rough, partly warty; unramified ends dark ochre to yellow with rusty spots; tips somewhat brighter ochre; older parts dark ochre to brownish.

Anatomical characters of surface.

Outer surface of mantle. — (Figs. 1b, c; 3a–c; 4f) Plectenchymatous, densely woven like a net formed by unbranched and multiple-branched hyphae; hyphae 2–4 µm in diam.; cell-wall inconspicuous; hyphae yellowish from membranal and plasmatic pigment. Some hyphae with droplet-like incrustations. Cystidia not found.

Inner layer of mantle. — (Fig. 3b) Plectenchymatous, loosely woven, multiple branched hyphae not as frequent as in outer surface, hyphae 2–4 µm in diam.; cell-wall inconspicuous; hyphae yellowish from membranal and plasmatic pigments.

Inner surface of mantle. — (Figs. 1d, 4e) More densely plectenchymatous than outer parts of mantle, with patches of shorter cells somewhat resembling epidermal cells; hyphae 3–5 µm in diam.

Rhizomorphs. — (Figs. 2b, d; 3d–f; 4b) Growing out of from mycorrhizae at a flat angle; running along surface of mantle; diameter up to 150 µm; hyphae of rhizomorphs differentiated in three types; outer hyphae: 3–4 µm in diam.; distance between septa 12–25 µm; cell-wall up to 1.5 µm thick, reddish brown; contents of hyphae yellowish from plasmatic pigments; inner hyphae: 3–5 µm in diam.; distance between septa 10–25 µm; cell-wall up to 0.5 µm; yellowish from membranal and plasmatic pigments; central hyphae: 5–18 µm in diam.; distance between septa (8–)10–25 µm; cell-wall up to 0.5 µm; yellowish from membranal and plasmatic pigments.

Anatomical characters observed in cross sections (Figs. 3d; 4c, d).

Mantle. — In outer part plectenchymatous, 20–45 µm thick; two layers recognizable, divided by a layer of collapsed residues of calyptra cells: hyphae in outer regions loosely woven, in inner parts of mantle more or less compact; in outer layer of mantle hyphae radially 2–3 µm, tangentially 5–10(–20) µm; in inner layer of mantle hyphae radially 3–5 µm, tangentially 4–10(–12) µm.

Tannin cells. — Very rare, tangentially elongate, heavily compressed, in one row, tangentially 5–25 µm, radially 4–10 µm; TCt = 13 µm; TCq 2.2.

Cortical cells. — Roundish, in 4 rows, tangentially (10–)15–35(–60) µm, radially (5–)20–35(–50) µm; CCt = 25 µm; CCq 1.1.

Hartig net. — (Figs. 2c, 4c) Number of rows of hyphae around cortical cells: 1; around Tannin cells not found (the very rare Tannin cells are embedded in inner part of mantle); Hartig net hyphae round to radially elongated, (2–)3–5(–7) µm thick; Hartig net often reaching endodermis.

Anatomical characters observed in median-longitudinal sections (Fig. 4a).

Hyphae of mantle. — Radially 2–5(–6) µm, tangentially 3–5(–10) µm; Tannin cells irregularly compressed, radially 4–10 µm, tangentially 35–70 µm; TCt = 53 µm; TCq 7.

Cortical cells. — More or less round, a few radially elongated ovoid; radially (5–) 20–35(–50) µm; tangentially (10–)15–25(–30) µm; CCt = 20 µm; CCq 0.8.

Hartig net. — Of palmetti type; lobes 2–3 µm broad (Fig. 2c).

Colour-reactions in different reagents.

Acetic Fuchsine: –; Aniline: dark brown drops in hyphae; Brilliant Cresyl Blue: –; Cotton Blue: –; Formaline 40%: –; Guaiac: –; Iron-sulfate: –; KOH: –; Melzer's reagent: –; Phenol: –; Phenol-Aniline: –; Sulfovanillin: cell-walls brown, not becoming red.

Autofluorescence (Figs. 4b, d–f).

Whole mycorrhizae: UV 254 nm: –; UV 366 nm: –. Hyphae of mantle and Hartig net: (observed in lactic acid) UV-filter 340–380 nm; outer layer of mantle and Hartig net only slightly yellow; inner layer of mantle bright yellow, with a reddish tinge in the triangular areas were hyphae of mantle penetrate in between outermost cortical cells; blue-filter (450–490 nm): all parts of mantle yellow; green-filter (530–560 nm): all parts of mantle pale reddish. Rhizomorphs: (observed in lactic acid) UV-filter 340–380 nm: outer hyphae: yellow to brownish red; inner and central hyphae pale blue; blue-filter (450–490 nm): outer hyphae: dark yellow to brownish; inner and central hyphae very pale yellow; green-filter 530–560 nm: –.

Staining of nuclei (Fig. 2a).

Two nuclei per cell (method: aceto-carmine); nuclei lying closely together (distance mostly 5–10 µm), round, 1.5–2.5 µm in diam., without siderophilous granulation.

Material studied and method of identification.

Reference specimen: Germany, Bavaria, Kelheim, Siegenburg, pine-forest, 25.9.1986 (fruit-body and mycorrhizae UD-50, both in M); there were direct connections between fruit-body and mycorrhizae via rhizomorphs; determination of fruit-body with Jülich (1984).

MYCORRHIZAE OF RHIZOPOGON LUTEOLUS IN LITERATURE

Pachlewski & Pachlewska (1974) synthesized ectomycorrhizae of *R. luteolus* on *Pinus silvestris* seedlings in agar-media using a pure fungal culture. They described the mycorrhizae of *R. luteolus* as frequently dichotomously furcate and often coralloid with shortened and thickened ends. The colour is given as creamy white to rusty brown. Moreover they mentioned a secretion that turns from colourless to brownish and an indistinctly two-layered mantle 30 to 60 µm thick. The Hartig net is described as well developed and reaching the endodermis.

With exception of the secretion, all features observed by Pachlewski & Pachlewska can be corroborated in the present study. Two points are noteworthy: (i) mycorrhizae described in the present study are merely yellowish to reddish brown; (ii) the mantle of the here described mycorrhizae is distinctly two-layered, an observation confirmed by autofluorescence data. Both differences might be due to the more advanced age of the mycorrhiza here described.

Malajczuk & Cromack (1982) identified the mycorrhizae of *R. luteolus* on *Pinus radiata*, but did not describe them. They found calcium-oxalate deposits on the hyphae. Such crystals could not be found in our specimen. According to Malajczuk & Cromack this could be due to different soil-conditions.

Baxter (1928) identified but did not characterize the mycorrhizae of *R. luteolus* on *Pinus silvestris*.

MYCORRHIZAE OF THE GENUS RHIZOPOGON IN LITERATURE

Noteworthy is the description of the mycorrhizae of *R. vinicolor* A.H. Smith on *Pseudotsuga menziesii* given by Zak (1971). *Rhizopogon vinicolor* forms a tubercle-like mycorrhiza with differentiated rhizomorphs. Rhizomorph organisation is like that of *R. luteolus* as characterized above. In addition Zak found that the envelope of the tubercles consisted of hyphae similar to the outer hyphae of rhizomorphs. The colour of the rind of the tubercle of *R. vinicolor* turned green, the colour of the mantle and of the hyphae inside the tubercles turned a pale pink in KOH. Pure culture synthesis of *R. vinicolor* with *Pseudotsuga menziesii* gave a pinnate mycorrhiza without ensheating fungal mantle.

Short descriptions of mycorrhizae of *R. subsalmonius, R. parksii, R. colossus* on *Abies concolor* and *R. villosulus* on *Pseudotsuga menziesii* are given by Acsai (1983) but his descriptions are too concise to be comparable with the description above or with that of Zak (1971).

The mycorrhizae of *R. rubescens* Tul. on *Pinus silvestris* have been described by Fontana & Centrella (1967). These mycorrhizae should have a two-layered mantle with the outer part loosely and the inner part more densely woven; a 24 to 32 μm thick mantle and 1.6 to 3.2 μm wide hyphae; emanating hyphae without clamps and with incrustations. Identification was based on the analogy of hyphal features in fruit-bodies, pure-culture, and mycorrhizae.

The characteristics of a pure culture-mycorrhiza of *R. rubescens* on *Pinus silvestris*, described in Pachlewski & Pachlewska (1974) corroborate the description of Fontana & Centrella (l.c.).

Baxter (1928) identified the mycorrhiza of *R. rubescens* on *Pinus silvestris* by tracing connecting hyphae between fruit-body and mycorrhizae but did not give descriptions.

Totten (1923) described a parasitic species of *Rhizopogon, R. parasiticus* Coker & Totten (Smith & Zeller, 1966), which absorbs completely the root of its host, which is entirely enclosed by a peridium of the species mentioned. A drawing of a longitudinal

section shows a two-layered mantle and a well-developed Hartig net of the palmetti type. The two layers of the mantle are divided by small compressed cells. This arrangement strikingly resembles the mycorrhizae described in the present publication. Unfortunately Totten (l.c.) gave no descriptions or drawings of details of the rhizomorphs although he obviously had seen them.

CONCLUSION

Summarizing the descriptions discussed the following features may be considered as common for the mycorrhizae of the genus Rhizopogon characterized up to now: (i) a plectenchymatic, two-layered mantle, (ii) highly differentiated and thick rhizomorphs, (iii) incrustations at least at some hyphae. The fact, that the mycorrhizae of *Rhizopogon luteolus* do not show a colour-reaction to KOH can be a special character of *R. luteolus*, because fruit-bodies of this species do not show this reaction either, whereas fruit-bodies of other species of this genus show reactivity to KOH (Smith & Zeller, 1966).

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LEGENDS

Fig. 1. *Rhizopogon luteolus*. — a. Habit of mycorrhizae. — b. Outer surface of mantle with multiple branched and unbranched hyphae forming hyphal net. — c. Outer surface of mantle at very tip of mycorrhiza. — d. Inner surface of mantle with elongate and epidermoid cells. (All figs. from UD-50.)

Fig. 2. *Rhizopogon luteolus*. — a. Nuclei of emanating hyphae and rhizomorphs stained with acetocarmine. — b. Rhizomorph organization as seen in longitudinal section; young rhizomorph without ensheating, thick-walled hyphae. — c. Hartig net; plan view of tangential section of mycorrhiza showing palmetti type. — d. Cross section of thick rhizomorph with thick-walled ensheathing hyphae; thin-walled inner hyphae enclosing thin-walled central hyphae with large lumena. (All figs. from UD-50.)

Fig. 3. *Rhizopogon luteolus*. — a. Outer surface of mantle with multiple branched and unbranched hyphae forming a net. — b. Plan view of middle layer of mantle, 10 μm deeper than 'a'. — c. Inner surface of mantle with elongate and epidermoid cells. — d. Young rhizomorph still embedded in outer layer of mantle. — e. Cross section of rhizomorph. — f. Thick rhizomorph; central hyphae with diameter up to 18 μm. (All figs. from UD-50; bar represents 10 μm.)

Fig. 4. *Rhizopogon luteolus*. — a. Longitudinal section of mycorrhiza from mantle to Hartig net and first row of cortical cells. — b. Longitudinal section of a rhizomorph with autofluorescence bright in outer hyphae and faint in inner part of rhizomorph (description of colours of autofluorescence see text). — c. Cross section of mycorrhiza with Hartig net hyphae and compressed calyptra cells dividing mantle into two layers. — d. Cross section of mycorrhiza showing autofluorescence; same details as 'c', but in addition outer and inner part of mantle show different degree of brightness of autofluorescence; notice bright autofluorescence of triangle-shaped tannin cell surrounded by hyphae of inner part of mantle. — e. Autofluorescence of inner surface of mantle. — f. Autofluorescence of outer surface of mantle. (All figs. from UD-50; bar represents 10 μm.)

Fig. 1

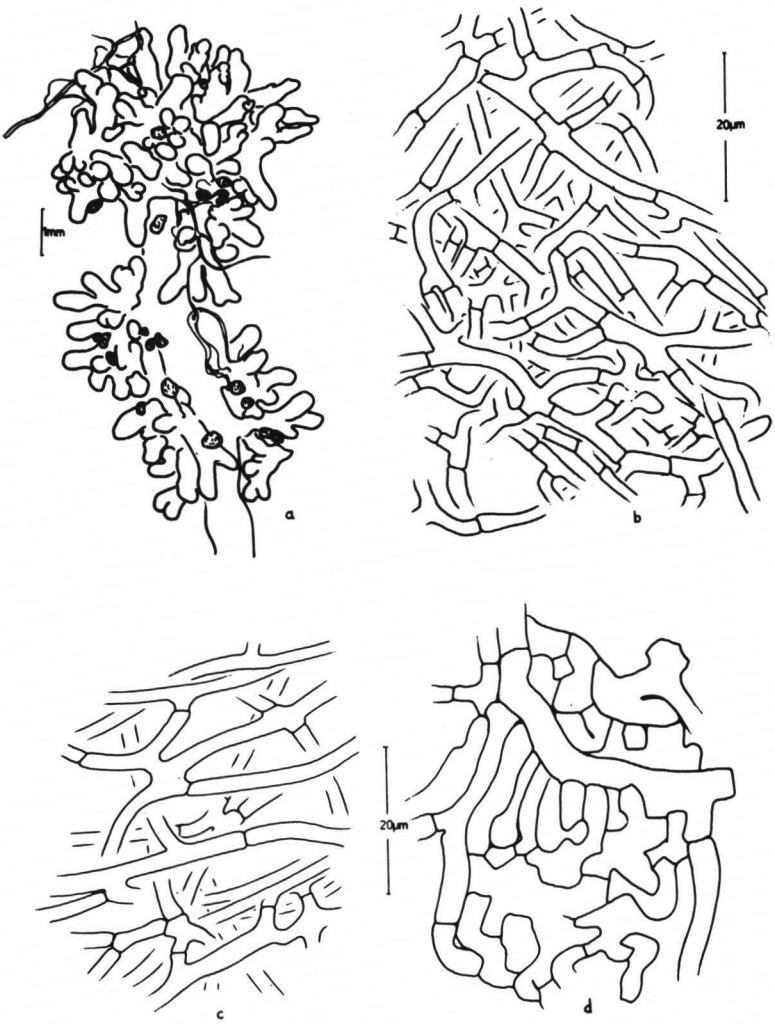

Fig. 2

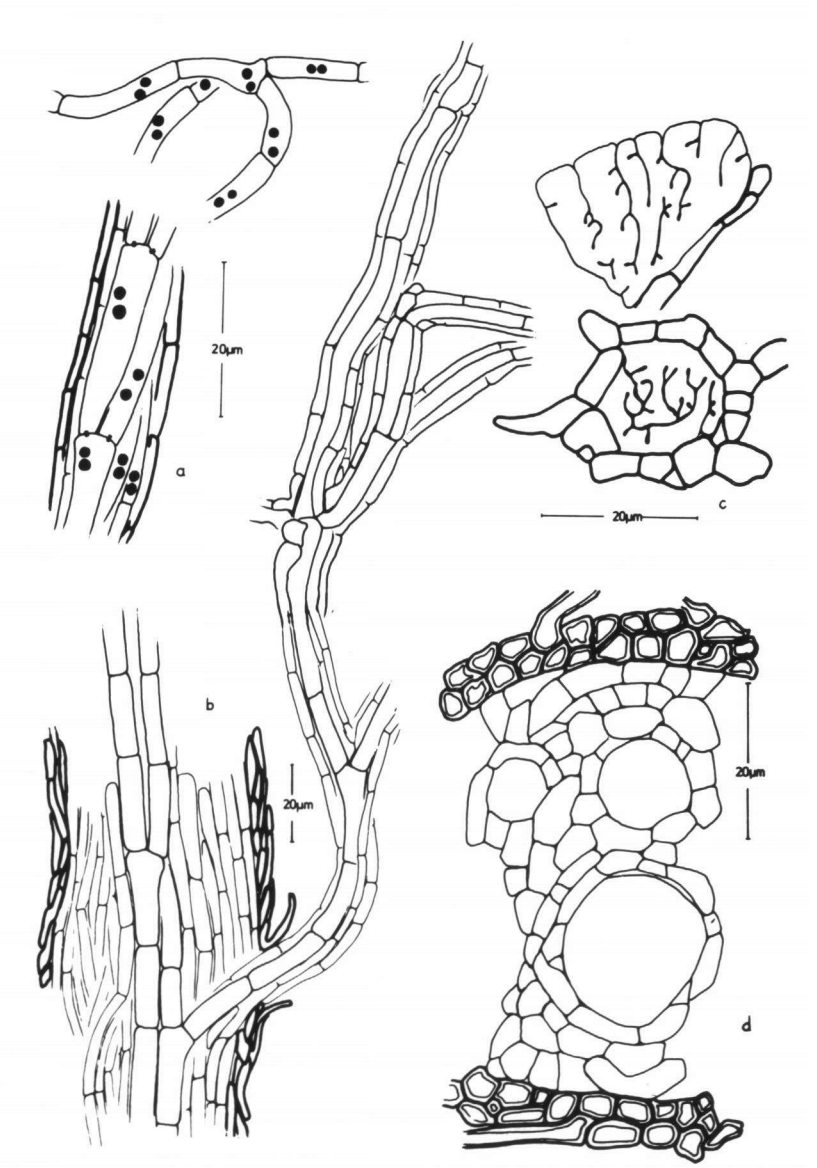

Fig. 3

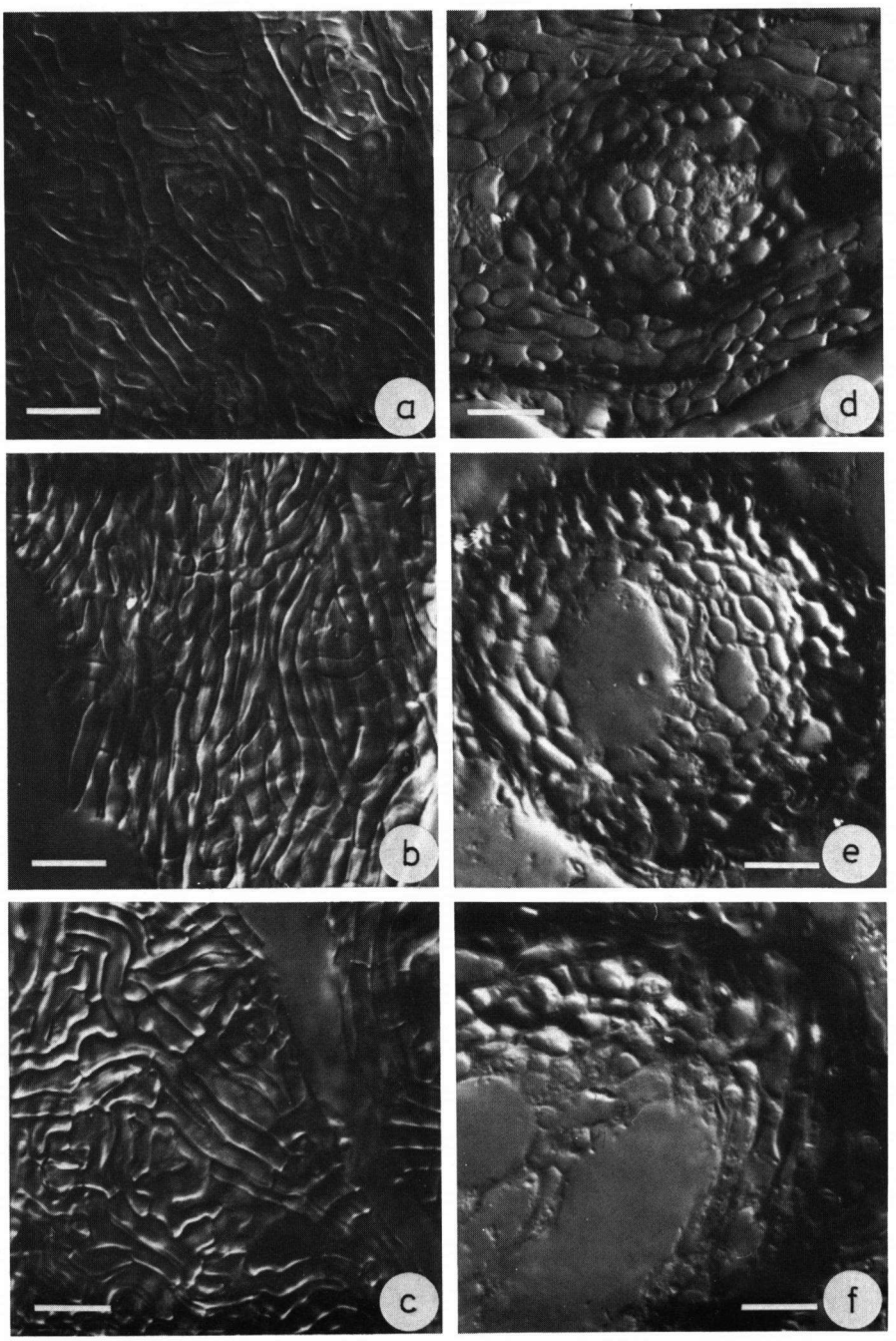

Fig. 4