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ORIGINAL PAPER

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Mycorrhiza of the host-specific *Lactarius deterrimus* on the roots of *Picea abies* and *Arctostaphylos uva-ursi*

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Abstract The ectomycorrhizal basidiomycete species *Lactarius deterrimus* Gröger is considered to be a strictly host-specific mycobiont of *Picea abies* (L.) Karst. However, we identified arbutoid mycorrhiza formed by this fungus on the roots of *Arctostaphylos uva-ursi* (L.) Spreng. in a mixed stand at the alpine timberline; typical ectomycorrhiza of *P. abies* were found in close relation. *A. uva-ursi* is known as an extremely unspecific phytobiont. The mycorrhizae of both associations are described and compared morphologically. The mycorrhiza formed by *L. deterrimus* on both *A. uva-ursi* and *P. abies* show typical ectomycorrhizal features such as a hyphal mantle and a Hartig net. The main difference between the mycorrhizal symbioses with the different phytobionts is the occurrence of intracellular hyphae in the epidermal cells of *A. uva-ursi*. This emphasizes the importance of the plant partner for mycorrhizal anatomy. This is the first report of a previously considered host-specific ectomycorrhizal fungus in association with *A. uva-ursi* under natural conditions. The advantages of this loose specificity between the fungus and plant species is discussed.

Keywords *Arctostaphylos uva-ursi* · *Picea abies* · *Lactarius deterrimus* · Arbutoid mycorrhiza · Ectomycorrhiza

Introduction

Sheathing mycorrhizae play an important role for plant development in subalpine protective forests (Moser 1956). The ecotone of the timberline in the Tyrolean Alps is characterized by a mixture of different conifers and extended areas of dwarf shrub communities. Ectomycorrhizal fungi are frequent in these areas forming two types of mycorrhiza: ectomycorrhiza on the roots of the main tree species (*Picea abies* (L.) Karst., *Pinus cembra* L., *Larix decidua* Mill.), and arbutoid mycorrhiza that are typical for *Arctostaphylos uva-ursi* (L.) Spreng. (bearberry) which is the only component of the dwarf shrub community forming ectotrophic mycorrhiza.

A. uva-ursi is known to form arbutoid mycorrhiza with a wide range of ectomycorrhizal fungi (Molina and Trappe 1982). The ability of *A. uva-ursi* to form mycorrhiza with ectomycorrhizal host-specific fungi has only been shown in synthesis experiments (Zak 1976; Molina and Trappe 1982). No observations on such an association have been made in nature. Records of ectomycorrhizal fruit bodies associated with bearberry strengthen the presumption that this plant is able to form mycorrhiza with fungi regarded to be specific for other host plants in nature (Acsai and Largent 1983a; Watling 2002).

Austrian protective forests in the subalpine area are known to be rich in fungal species (Peintner 1995). Few of these fungi are known as strictly specific for distinct tree species. *Lactarius deterrimus* Gröger belongs to the most frequent ectomycorrhizal partners of *P. abies* in the Alps (Engel and Friedrichsen 1971; Rücker et al. 1990; Peintner 1995). Field observations (Moser 1983; Heilmann-Clausen et al. 1998) and descriptions of selected ectomycorrhiza (Agerer 1986; Münzenberger et al. 1986) indicate a high degree of specificity for this fungal species.

During our extensive investigation of mycorrhiza of *A. uva-ursi*, we collected basidiomes of *L. deterrimus* in close relation with both Norway spruce and bearberry. This fungus is generally regarded as strictly host-specific to Norway spruce roots (Heilmann-Clausen et al. 1998). Our microscope analyses have shown that this fungus colonized the fine roots of both plants. In this study we describe and compare the features of arbutoid mycorrhiza on *A. uva-ursi* and ectomycorrhiza on *P. abies* formed by *L. deterrimus* in nature. Host-specificity of mycorrhizal

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fungi and the influence of *A. uva-ursi* on the establishment of afforested trees are discussed.

Materials and methods

Study site

The samples were taken in the area of Haggen (Sellrain valley, Tyrolean Alps, Austria (N 47°13′, E 011°03′)) in August 2002. The southern exposed slope at 2000 m above sea level (a.s.l.) is characterized by a sparse, middle aged stand of *P. abies*, *P. cembra* and *L. decidua*, which developed after decades of human deforestation. In between this tree population a *Junipereto–Arctostaphyletum* community is widespread; the dominant components are: *A. uva-ursi*, *Juniperus communis* L. *var. alpine* Sm. and *Vaccinium vitis-idaea* L. (Neuwinger 1972).

Sampling, preparation and characterization

Soil samples with roots of *A. uva-ursi* and *P. abies* were taken by cutting out a 10 cm^3 block of soil underneath the fruit bodies of *L. deterrimus*. Fine roots of *A. uva-ursi* and *P. abies* were dissected carefully under a stereomicroscope (Nikon SMZ-U). Mycorrhiza connected to the fruit bodies by fungal mycelium were selected and observed fresh according to Agerer (1991). Macroscopical characters including color, branching, features of the mantle surface and emanating elements, were noted. Hand sections and thin sections (with a Reichert-Jung Cryocut 1800 freeze microtome) of the fine roots of *A. uva-ursi* and *P. abies* were made. Mantle layers were scraped to judge plectenchymatous or pseudoparenchymatous parameters. Microscopic features, such as plan views of mantle layers, cross-sections, longitudinal-radial sections of mycorrhizal tips and characteristics of emanating elements, were assessed with a LEITZ Diaplan research microscope equipped with (interference) phase contrast objective. Additionally, sections of mycorrhizal root tips and scraped mantle layers were stained in different reagents and observed in the microscope. A digital camera (NIKON COOLPIX 995) was used for photographic documentation. Voucher material of both mycorrhizal types was fixed in formaldehyde: ethanol 70%: acetic acid, 5:90:5 (FEA), stored at 4°C according to Agerer (1991) and deposited in the Mycological Collection of the University of Innsbruck; IB20020100 (*P. abies*) and IB20020102 (*A. uva-ursi*) (IB).

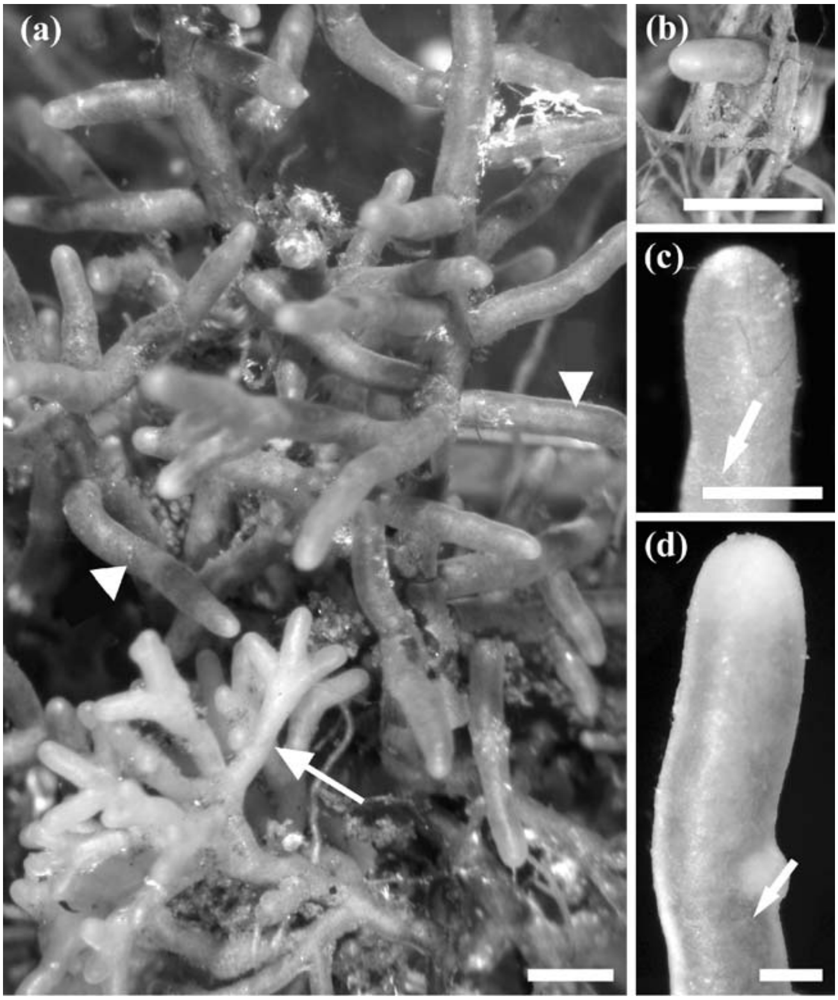

Fig. 1 Macroscopic appearance of *Lactarius deterrimus* mycorrhizae; **a** Arbutoid mycorrhiza of *Arctostaphylos uva-ursi* (*arrow*) and ectomycorrhiza of *Picea abies* (*arrowhead*) in close relation (bar 1 mm) **b** Rhizomorphs on fine roots of *A. uva-ursi* (bar 1 mm); **c** Higher magnification of *A. uva-ursi* mycorrhiza, note lactiferous hyphae (*arrow*; bar 0.25 mm); **d** Detail of *P. abies* mycorrhiza, note lactiferous hyphae (*arrow*; bar 0.25 mm)

Results

Macroscopic features of *Lactarius deterrimus* mycorrhiza

The ectomycorrhizal anatomy of *L. deterrimus* on *P. abies* has been described in detail by Agerer (1986, 1987–2002). For comparison with the arbutoid mycorrhiza, only the main characters will be mentioned here. Ectomycorrhizal systems of *P. abies* are irregularly branched and pyramidal (Fig. 1a). Axes are straight to slightly bent. The colour of mycorrhiza is ochre-orange in young fine root tips changing to greenish with aging. The mantle surface is smooth and shiny. Lactiferous hyphae can be seen at higher magnification (Fig. 1d). Rhizomorphs are rarely observed; they appear in the same color as the mycorrhizae.

Arbutoid mycorrhiza macroscopically resembles the mycorrhiza of Norway spruce. The mycorrhiza on *A. uva-ursi* is regularly trilobate, occurring as dense clusters (Fig. 1a). They are about one third smaller in diameter than the *P. abies* mycorrhiza. The colour of arbutoid mycorrhiza appears a little brighter than that of the ectomycorrhiza, but it can be described as ochre-orange with a tendency to green in older tips. The surface appears smooth and shiny. Lactiferous hyphae can be observed at a higher magnification (Fig. 1c). Rhizomorphs are rare; they appear in the same colour as the mantle (Fig. 1b).

Microscopic features of *L. deterrimus* mycorrhiza on the roots of Norway spruce

Longitudinal sections of *P. abies* mycorrhizae show a well developed mantle layer, between 25 and 30 µm thick (Fig. 2a). The hyphae forming the mantle are packed very densely. The hyphae of the Hartig net penetrate between the cortical cells (arrows). The first plant cell row contains tannins and, thus, appears darker than the other cells. The

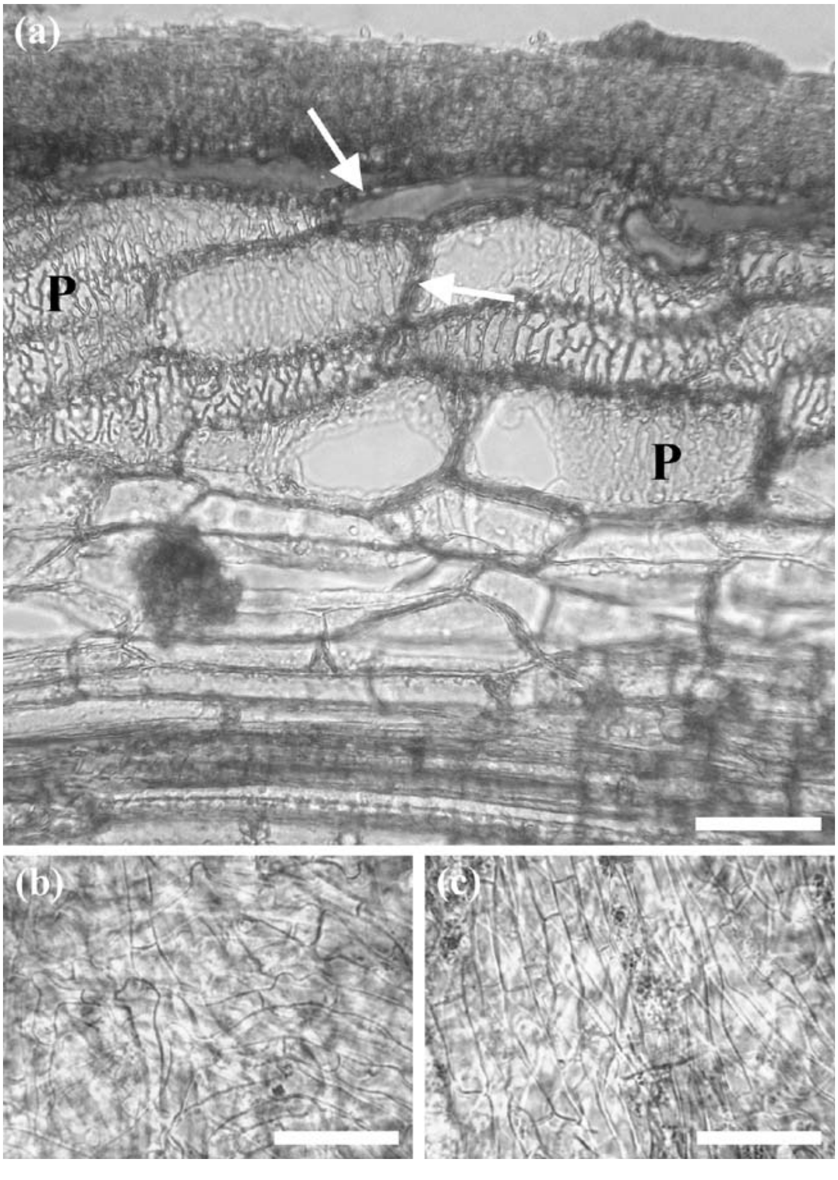

Fig. 2 Light microscopy of *Lactarius deterrimus–Picea abies* mycorrhiza. **a** Longitudinal section of root tip (bar 30 µm). Hyphae belonging to the Hartig net (*arrows*) can be seen as a Palmetti structure about four cell rows deep (*P*); **b** Inner mantle surface (bar 10 µm); **c** Outer mantle surface (bar 10 µm)

Hartig net in plan view can be recognized in the cortical cell layers as a finger-like formed palmetti structure (P). The hyphae of the Hartig net penetrate between the first four cortical cell rows as additional cross-sections have shown. No intercellular hyphae can be observed in deeper cell rows up to the endodermis of the root. The plan view of the inner mantle layer shows an irregular plectenchymatous structure (Fig. 2b). The outer mantle layer is plectenchymatous (Fig. 2c). The mantle hyphae are approximately 4 μm thick.

Microscopic features of *L. deterrimus* mycorrhizae on the roots of *A. uva-ursi*

Sections of *A. uva-ursi* fine roots show a typical arbutoid anatomy (Fig. 3a). The mantle layer is well developed, between 20 and 30 μm in diameter. Hyphae penetrate between the epidermal cells, frequently branching to form a paraepidermal Hartig net (arrow). The plan view of the Hartig net shows the Palmetti structure (P). Furthermore, hyphae from the Hartig net penetrate the epidermal cells producing hyphal coils, filling the whole cell volumina. Longitudinal sections show that the penetration starts some cells behind the tip of the root. The plan views of the inner (Fig. 3b) and outer mantle layers (Fig. 3c) appear similar to the ectomycorrhiza on *P. abies*. The inner hyphae are irregularly branched and bent, likewise forming plectenchymatous structures. The outer mantle layer is more regularly plectenchymatous. The hyphae are approximately 4 μm thick.

Microchemical reactions in different reagents

Microchemical reactions were observed only in the plan view of scraped mantle layers. In the mantle layers of the arbutoid mycorrhiza and the ectomycorrhiza, lactiferous hyphae were stained dark red with sulfo-vanillin. Other chemicals (KOH 10%, Melzer's reagent, cotton blue) showed no reaction.

Discussion

The connection by fungal mycelium shows that *L. deterrimus* is the fungal partner of both symbioses. Lactiferous hyphae on both root types and the plan views of the mantle layers confirm this report. Nevertheless, there are some structural determinations between arbutoid mycorrhiza and ectomycorrhiza formed by *L. deterrimus* due to the unequal plant partners. The most remarkable difference is the occurrence of intracellular hyphae in the epidermal cells of *A. uva-ursi*. Hyphae of the fungal symbiont penetrate epidermal cells of *A. uva-ursi* from the Hartig net and form coils that are typical of arbutoid mycorrhiza (Acsai and Largent 1983b; Münzenberger et al. 1992; Smith and Read 1997). Other differences are the

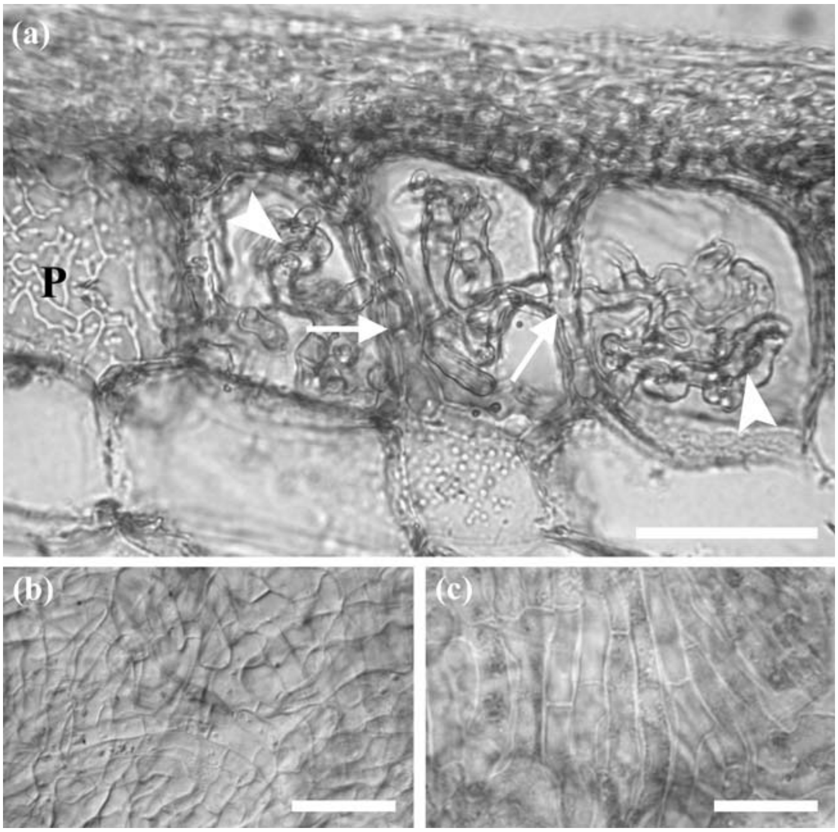

Fig. 3 Light microscopy of *Lactarius deterrimus*–*Arctostaphylos uva-ursi* mycorrhiza. **a** Longitudinal section of root tip (bar 30 μm). Hyphae from the paraepidermal Hartig net (*arrows*) penetrate epidermal cells and form hyphal coils (*arrowhead*). Hartig net shows the Palmetti structure (*P*) in plan view; **b** Inner mantle surface (bar 10 μm); **c** Outer mantle surface (bar 10 μm)

form of the Hartig net, branching pattern and colour. *L. deterrimus* hyphae penetrate between the cell rows of *P. abies* roots and branch to form the labyrinthine structure of the Hartig net (Fig. 2a). This character, called "cortical Hartig net", is common for gymnosperms (Scheidegger and Brunner 1995). In *A. uva-ursi* the Hartig net surrounds only the epidermal cell row (Fig. 3a), forming the "epidermal Hartig net", typically formed in angiosperms (Scheidegger and Brunner 1995; Smith and Read 1997). Angiosperms prevent further penetration of the hyphae by a suberin lamella, formed around the cells of the second cortical layer (Smith and Read 1997). This lamella has already been described for arbutoid mycorrhiza (Münzenberger et al. 1992). The function of the Hartig net is to enlarge the contact surface between plant and fungus for a better nutrient transport (Scheidegger and Brunner 1995). Due to the limitation of fungal penetration caused by the barrier of the suberin lamella in the second cell row, this contact surface is reduced in angiosperms compared to gymnosperms. The arbutoid mycorrhiza formed on *A. uva-ursi* is trilobate, whereas, those on *P. abies* are of an irregular pyramidal shape; the ectomycorrhiza appear darker than the arbutoid mycorrhiza though this may be related to different ages of the mantle layers (Ingleby et al. 1990).

A. uva-ursi has been described to form mycorrhiza with a wide range of fungi in vivo and in vitro (Mejstrik and Hadac 1975; Zak 1976; Molina and Trappe 1982; Acsai and Largent 1983b; Molina et al. 1997). Furthermore, *A. uva-ursi* has been reported repeatedly to be a refuge plant for mycorrhizal fungi in different forests (Acsai and Largent 1983a; Horton et al. 1999; Hagerman et al. 2001). However, only ex situ synthesis experiments have clearly shown yet that *A. uva-ursi* can also form mycorrhiza with fungi regarded to be strictly host-specific, such as *Lactarius* (*L. sanguifluus* and *L. deliciosus* related to *Pinus* species) or *Suillus* species (Zak 1976; Molina and Trappe 1982). No associations of *A. uva-ursi* with fungi that are specific to other host plants have been reported in nature before this study. The extreme unspecificity of *A. uva-ursi* as mycorrhizal host plant (Zak 1976; Molina and Trappe 1982) could be explained by its wide distribution, in different habitats, such as coastal sand dunes, mudflows and glacial outwashes (Molina and Trappe 1982). As such, *A. uva-ursi* must have developed a nonselective strategy concerning fungal partners because every habitat can have a different composition of fungal species.

In the Tyrolean Alps, *A. uva-ursi* occurs mainly between subalpine forests and alpine dwarf shrub zones above the actual timberline where fungal diversity is high and where *A. uva-ursi* has been reported to be closely related with many ectomycorrhizal fungi (Göbl and Ladurner 2000; Mühlmann 2004). In these areas, natural forests have often disappeared due to human influence (Schiechtl 1970), and extreme climatic conditions make successful establishment of replanted seedlings difficult. Here, *A. uva-ursi* could be an important nurse plant for the re-establishment of *P. abies* in the deforested areas of the Tyrolean Alps: (1) as a refuge plant for ectomycorrhizal fungi as proposed by Horton et al. (1999) and Hagerman et al. (2001), or (2) through ectomycorrhizal fungi nutrient transfer between *A. uva-ursi* and reforested trees as suggested by Molina and Trappe (1982) and demonstrated for ectomycorrhizal trees (Simard et al. 1997).

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