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Anatomical aspects of field ectomycorrhizas on *Polygonum viviparum* (Polygonaceae) and *Kobresia bellardii* (Cyperaceae)

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Abstract Root systems of the herbaceous species *Polygonum viviparum* and *Kobresia bellardii* were excavated from an alpine site in the Rocky Mountains, Colorado, and processed for microscopic examination. Several ectomycorrhizal morphotypes were present on root systems of both species; *K. bellardii* often had complex clusters of mycorrhizal roots present. A mantle and Hartig net were present on all mycorrhizal root tips processed. The Hartig net was confined to the epidermis, and the parenchyma cells of this layer were radially elongated, vacuolated and contained densely staining inclusions. Intracellular hyphae and structures typical for vesicular-arbuscular mycorrhizas were never observed. Both herbaceous species, therefore, had ectomycorrhizal associations comparable to those described for woody angiosperm species.

Key words *Kobresia* · *Polygonum* · Ectomycorrhiza · Alpine · Anatomy

Introduction

The occurrence of ectomycorrhizas on herbaceous plant genera has been recognized for almost a century but has rarely been documented cytologically. Observations of members of the Polygonaceae by Hesselman (1900) indicated the presence of a mantle and Hartig net on *Polygonum viviparum* L. roots. Later, Costantin and Magrou (1926) confirmed the findings of Hesselman with root material from France and Italy, Peyronel (1930) supported the observations on *P. viviparum*, and Chernyayeva (1960) documented ectomycorrhizas on *P. weyrichii* Fr. Schm. More recently, Zak (1973) indicated a possible ectendomycorrhizal association between a *Cortinarius* species and *P. paronychia* Cham. and Schlecht, but did not elaborate further. Fontana (1977) mentioned 16 fungal morphotypes present on *P. viviparum*, and described the Hartig net enveloping and penetrating into a single layer of lengthened cells, a behavior similar to ectendomycorrhizas. Lesica and Antibus (1986) listed *P. viviparum* as being predominantly ectomycorrhizal whereas *P. bistortoides* Pursh was associated with vesicular-arbuscular mycorrhizal fungi on acidic, crystalline soil sites in the Rocky Mountains. Blaschke (1991a) reported *P. viviparum* to be ectomycorrhizal in the Bavarian Alps in Germany, and the author provided diagrams of root systems and a low magnification picture of a short ectomycorrhizal root of *P. viviparum*; both endomycorrhiza and ectomycorrhiza were reported to be present in the same root system (Blaschke 1991b). Treu et al. (1996) reported that *P. viviparum* was ectomycorrhizal, whereas *P. bistorta* L. did not appear to be mycorrhizal in Denali National Park in Alaska.

The genus *Coccoloba*, a tropical member of the Polygonaceae with approximately 125 species (Brandbyge 1990), is also ectomycorrhizal (Kreisel 1970). The species *C. uvifera* (L.) L. was shown to have five distinct morphological types of ectomycorrhizas and appears to possess both a well-developed mantle as well as an epidermal Hartig net (Kreisel 1970).

Another herbaceous genus, *Kobresia*, in the Cyperaceae, was described by Fontana (1963) as being ectomycorrhizal. She distinguished three morphological types of ectomycorrhizas on *K. bellardii* (All.) Degl., one of which appeared to be formed by *Cenococcum* and to possess a typical fungal mantle and epidermal Hartig

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net. Haselwandter and Read (1980) confirmed the mycorrhizal status of both *K. myosuroides* (Vill.) Fiori and *Polygonum viviparum*, but did not provide anatomical details. In a survey of plants for mycorrhizal status on Ellesmere Island, a high arctic site (Kohn and Stasovski 1990), *K. myosuroides* was found to have an ectomycorrhizal association with Hartig net hyphae between epidermal cells and intracellular hyphae in some root samples.

In this study, we document the anatomy of *P. viviparum* and *K. bellardii* = *K. myosuroides* mycorrhizas collected from an alpine setting in the Rocky Mountains of Colorado.

Materials and methods

Site description and source of material

The site was located in the upper Green Lakes basin of North Boulder Creek, 40° 3.2′ N. Lat., 105° 37.5′ W. Long., at approximately 3500 m elevation and about 7 km west of the Mountain Research Station, University of Colorado Institute of Arctic and Alpine Research. Green Lakes basin is adjacent to the Niwot Ridge Biosphere Reserve and Long-Term Ecological Research (LTER) Site.

Plants were collected from wet, moist, and dry meadow vegetation communities (May and Webber 1982). Pieces of highly organic alpine sod (approximately $10 \times 10 \times 15$ cm) containing several plants of *P. viviparum* or *K. bellardii* were extracted from the soil in July 1989, transported to the Mountain Research Station and stored at 4 °C until processing. Plant roots were gently washed, removing soil and debris, and a selection of attached mycorrhizal roots (belonging to several morphological types) was removed with forceps and fixed for light microscopy.

Light microscopy and fluorescence microscopy

Turgid, apparently healthy, ectomycorrhizas were fixed in 2.5% glutaraldehyde in 0.10 M HEPES buffer (pH 6.8) at ambient temperature for 4 h, rinsed in buffer several times, dehydrated in a graded series of ethanol and embedded in gelatin capsules using LR White Resin (London Resin Company Ltd.) immediately after dehydration. Thick sections (1–1.5 μm) were cut with glass knives on an MT-microtome, stained for light microscopy with a solution of 1% (w/v) methylene blue, 1% (w/v) azure B and 1% (w/v) sodium tetraborate in distilled water, rinsed in distilled water, counterstained in 0.5% (w/v) aqueous basic fuchsin, air dried, mounted in immersion oil and viewed on a Leitz Orthoplan photomicroscope. More than five roots of each species at various stages of development were examined. Some sections of both species were also stained for polysaccharides using the acriflavine-HCl procedure outlined in Culling (1974). These sections were viewed and photographed on a Leitz SM-LUX microscope with an epi-illumination system using a broad-band ultraviolet excitation filter providing 340–380 nm wavelengths combined with barrier filter 400 K-430.

Results and discussion

Excavated root systems of *P. viviparum* had many mycorrhizal lateral roots attached to long roots emanating from small bulbils, structures which may be important propagules in cold climates (Bauert 1993) (Fig. 1a, b). Mantles of some ectomycorrhizas were light in colour (Fig. 1b), whereas others were dark and *Cenococcum*–like (Fig. 2). Root systems of *K. bellardii* had mycorrhizas which were either single to pinnate (Fig. 3), or sometimes in clusters with several distinct fungal morphotypes (Fig. 4).

Polygonum viviparum mycorrhizas had a mantle covering the root apex and initiated an Hartig net close to the apical meristem (Fig. 5) to form a distinct epidermal Hartig net between radially elongated epidermal cells. Epidermal cells immediately proximal to the apical meristem were vacuolated and had discrete dark deposits and sometimes a layer of dark material lining the cell wall (Figs. 5, 6). Most roots had 1–2 cortical cell layers and a very reduced root cap (Figs. 5, 8). Paradermal sections through the epidermis showed the spatial relationship between intercellular hyphae and epidermal cells forming a uniseriate Hartig net (Fig. 7); intracellular hyphae were not detected. Most tissues stained intensely for polysaccharides following treatment of sections with the fluorochrome, acriflavine-HCl (Figs. 8, 9). The inner layer of the mantle was especially intensely stained, perhaps indicating that the majority of stored polysaccharides are in this region (Fig. 8). Polysaccharides are frequently stored in mantles of various ectomycorrhizas (Herr and Peterson 1996). The Hartig net was also acriflavine-positive (Fig. 9).

Sections of *K. bellardii* mycorrhizas showed that the Hartig net developed in close proximity to a small meristem region (Fig. 10) and was located only adjacent to radially elongated epidermal cells (Figs. 10–12). The mantle consisted of several layers of compactly arranged hyphae (Figs. 10–12). Acriflavine-stained sections showed intense staining of root tissues, but particularly the inner mantle (Fig. 12).

Although the fungal symbionts were not identified, root systems of both *P. viviparum* and *K. bellardii* sam-

Fig. 1 **a** Bulbil (*) of *Polygonum viviparum* showing origin of adventitious roots (*arrowheads*) from which short, lateral roots originate. **b** Portion of *P. viviparum* root system showing mycorrhizal lateral roots (*arrowheads*)

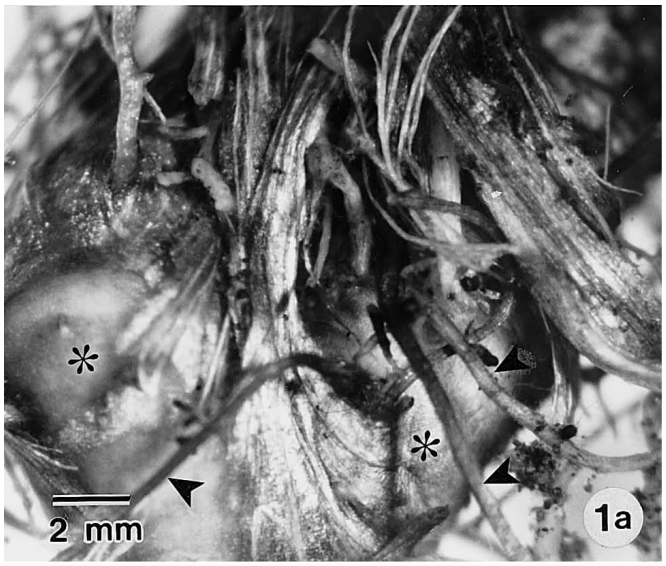
Fig. 2 An enlargement of a single mycorrhizal root of *P. viviparum* with a dark fungal mantle (*arrowhead*)

Fig. 3 Segments of root system of *Kobresia bellardii* with mycorrhizal lateral roots (*arrowheads*)

Fig. 4 A cluster of *K. bellardii* ectomycorrhizas. Several morphotypes are evident (*arrowheads*)

Fig. 5 Longitudinal section (LS) of *P. viviparum* ectomycorrhiza showing mantle (*), initiation of Hartig net (*arrowheads*) and radially-elongated epidermal cells (*E*)

Fig. 6 LS of *P. viviparum* ectomycorrhiza showing a uniseriate Hartig net (*arrowheads*) proximal to the apical meristem, and radially-elongated epidermal cells (*E*) with densely stained deposits lining some epidermal cells (*double arrowhead*) and as discrete bodies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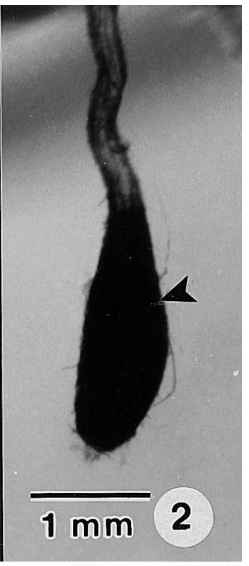
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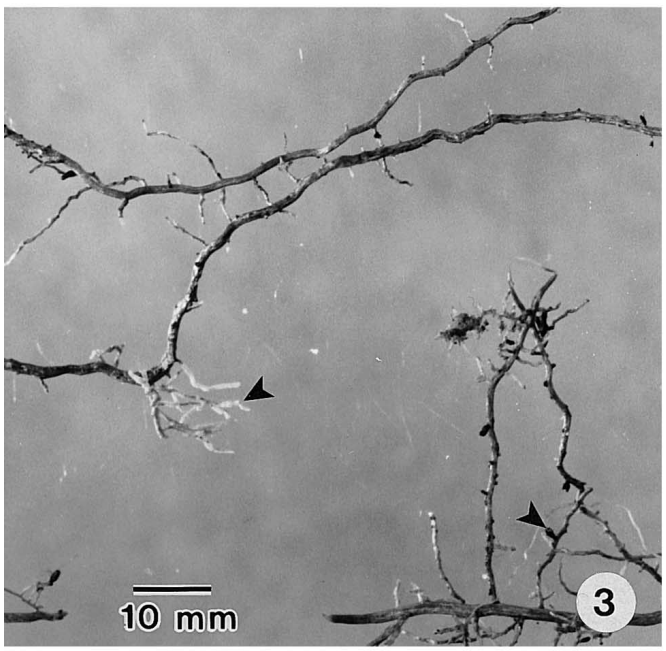
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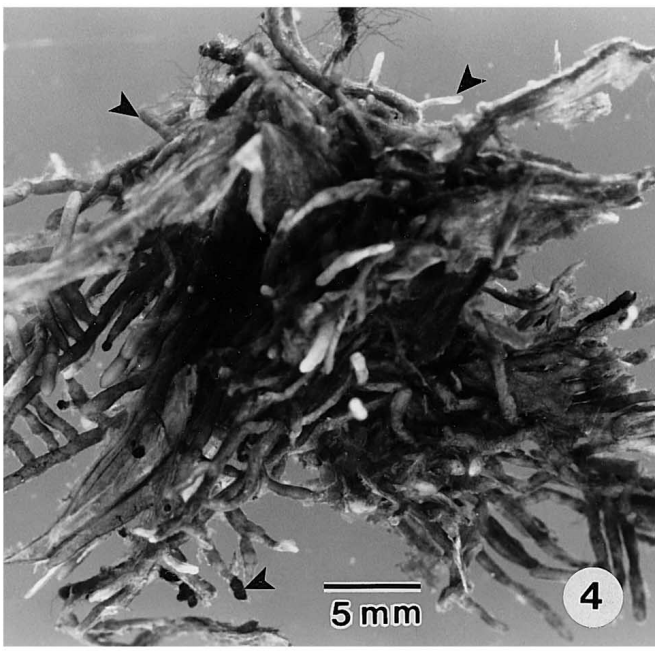
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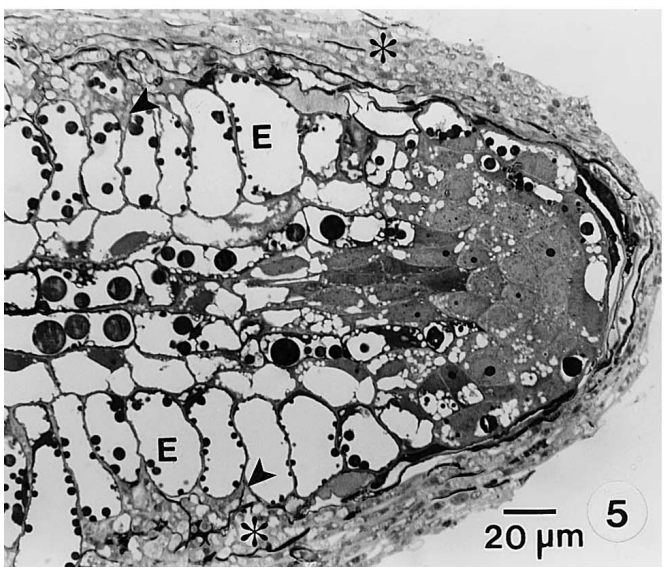
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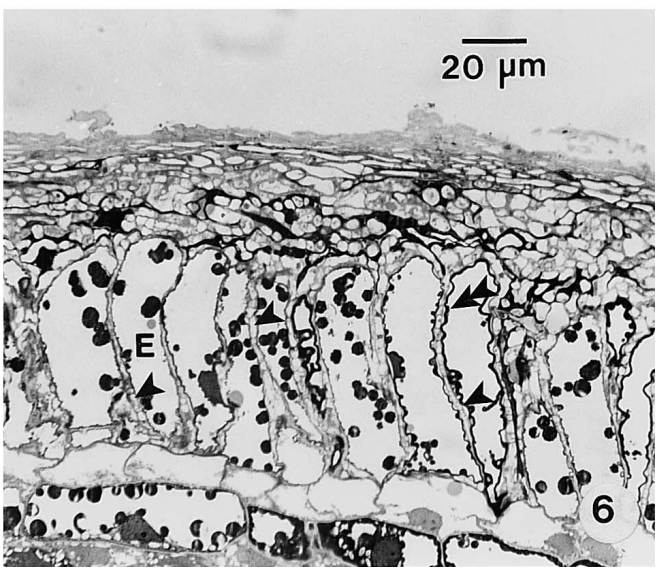
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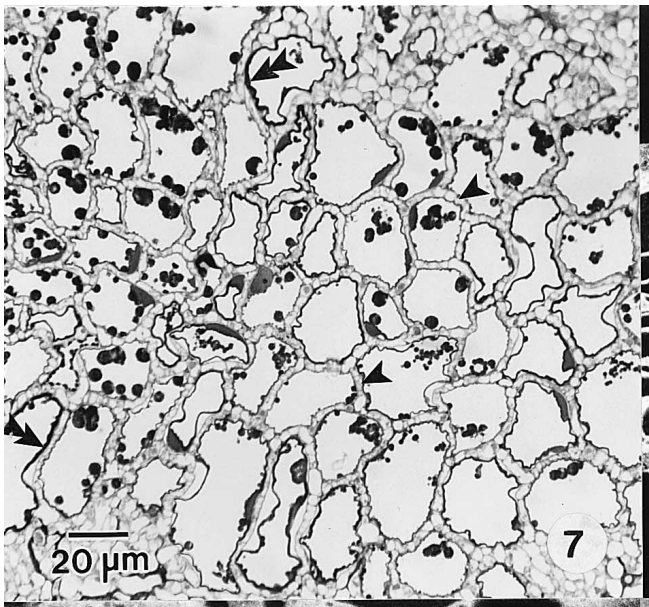
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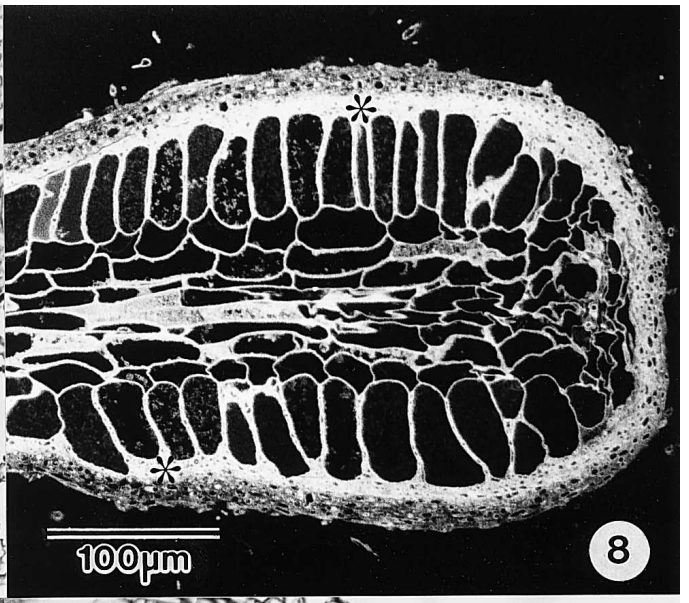
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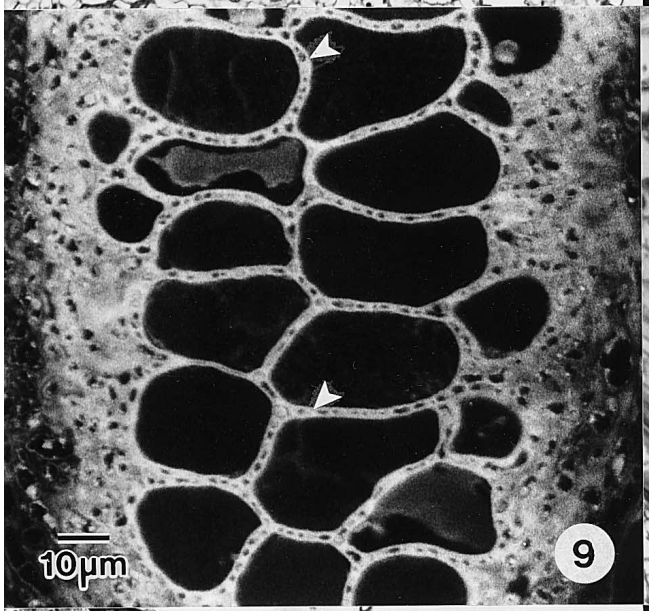
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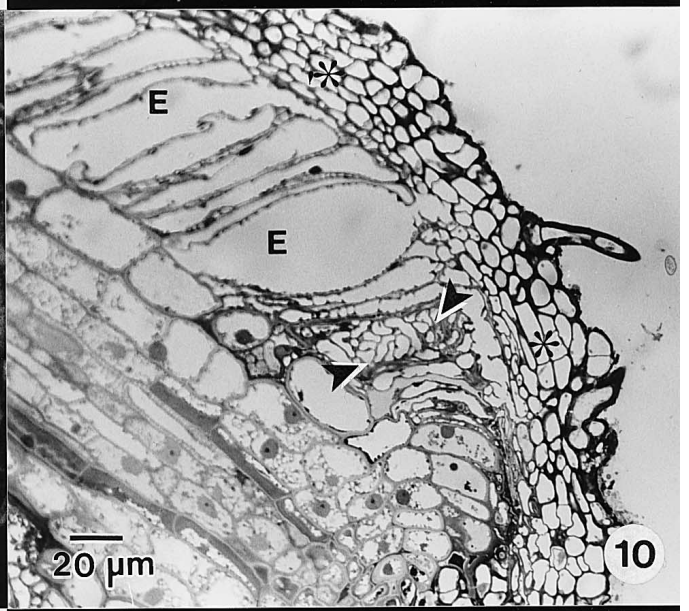
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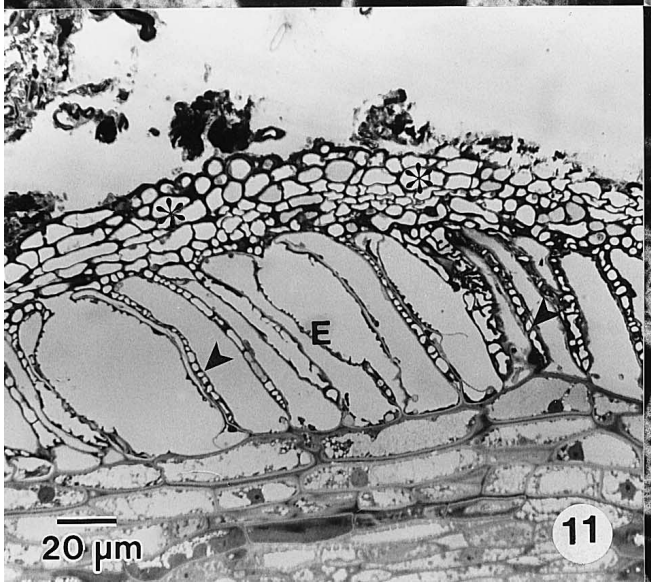
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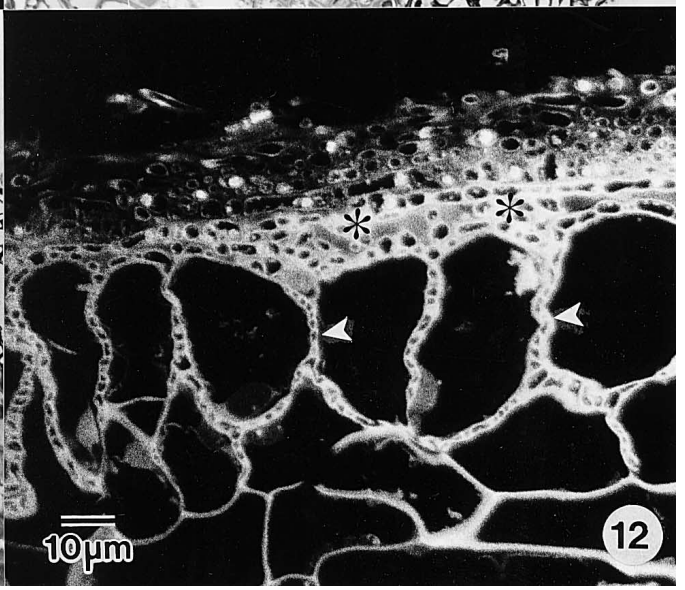
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pled from the Green Lakes / Niwot Ridge LTER site (>3500 m elevation) had several ectomycorrhizal morphotypes based on mantle colour and texture. *Cenococcum* colonization of *K. bellardii* was variable, ranging from <5 to <90% of the tips colonized. *P. viviparum* was generally abundantly ectomycorrhizal (>50% of the tips). Roots of this species are reported to exhibit also vesicular – arbuscular mycorrhizas or, rarely, only vesicular – arbuscular mycorrhizas (Luoma and Trappe unpublished data). We found no evidence of structures associated with vesicular-arbuscular mycorrhizas. Gardes and Dahlberg (1996) recently reviewed the diversity of fungal morphotypes found on several alpine plant species, and for the herbaceous species *P. viviparum* they noted that *Cenococcum*, *Amanita*, *Inocybe* and *Russula* had been reported as fungal symbionts. Based on mantle colour and the presence of coarse emanating hyphae, *Cenococcum* was likely a fungal symbiont on the *P. viviparum* plants collected in this study. Fontana (1977) mentioned 16 fungal morphotypes (one with *Cenococcum*, one with *Russula emetica* var. *alpestris*) present on *P. viviparum*. For *K. bellardii*, a *Cenococcum*–like morphotype was present at this site; Gardes and Dahlberg (1996) list only *Cenococcum* as a known symbiont with *Kobresia*, but again more than one morphotype appeared to be present at the Niwot Ridge site. PCR-based molecular methods could be used to identify fungal symbionts forming ectomycorrhizas with these two herbaceous species.

Polygonum viviparum and *K. bellardii* are herbaceous plant species, the former a dicotyledonous angiosperm and the latter a monocotyledonous angiosperm. Both, however, exhibited features characteristic of ectomycorrhizas of woody dicotyledonous angiosperms: an epidermal Hartig net associated with radially elongated epidermal cells and a moderately thick fungal mantle usually enclosing the root apex. The restriction of the Hartig net to the epidermis might be correlated to the presence of suberin in the walls of cells in the outer layer of cortex, as shown for *Eucalyptus pilularis* (Massicotte et al. 1987), but this has not been determined for the species in this study or in previous extensive surveys of angiosperm species (Perumalla et al. 1990; Peterson and Perumalla 1990).

None of the material examined showed intracellular hyphae and, therefore, an ectendomycorrhizal type of colonization was absent. This differs from observations by Fontana (1977) on *P. viviparum* and Kohn and Stasovski (1990) on *K. bellardii*, in which some intracellular hyphae were reported. This may reflect a difference in age of roots sampled, because it is known that senescing ectomycorrhizas may have cells invaded by hyphae (Thomas and Jackson 1979). There is speculation that some ectendomycorrhizas are simply a developmental variation of ectomycorrhizas manifested under conditions of physiological stress (Egger and Fortin 1988); this requires further research.

Several authors (Nespiak 1953; Bledsoe et al. 1990; Väre et al. 1992) found that ectomycorrhizas were not present on field-collected *P. viviparum* plants. Furthermore, in an extensive survey of plant species for mycorrhizal associations, Currah and Van Dyk (1986) examined *P. amphibium*, *P. douglasii* and *P. viviparum* from various sites in Alberta, Canada and also found that these three species lacked mycorrhizas of any type, but that *P. viviparum* did have dematiaceous surface hyphae. *P. viviparum* has been reported also to have vesicular-arbuscular mycorrhizal associations in collections from calcicole habitats (Blaschke 1991a,b).

The very mixed reports of the mycorrhizal status of *P. viviparum* may be related to the stage of plant development at which root samples were collected, variations in soil conditions, or the season in which collections were made. It would be of interest to test the plasticity of the interaction of this species with various mycorrhizal fungal symbionts under controlled conditions.

Temperature and the duration of the growing season are severe constraints for plant growth in alpine settings and, therefore, mycotrophic structures may be essential to the functioning and fitness of these plant species (Read and Haselwandter 1981). As interest in the nutrient status of plants at high altitudes grows (Körner 1989), an understanding of the mycotrophy of these plants remains an important and ecologically relevant goal, especially the roles of various fungal symbiotic associations in these stressed environments.

Fig. 7 Paradermal section through the epidermis of a *P. viviparum* ectomycorrhiza showing an uniseriate Hartig net (*arrowheads*). Some epidermal cells are lined with densely staining material (*double arrowheads*) and most have discrete densely staining bodies

Fig. 8 LS of a *P. viviparum* ectomycorrhiza showing a two-layered mantle; the inner layer (*) stains intensely for polysaccharides as shown by fluorescence microscopy of an acriflavine-stained section

Fig. 9 Paradermal section of a *P. viviparum* ectomycorrhiza stained with acriflavine and viewed with fluorescence microscopy, showing an obvious uniseriate Hartig net (*arrowheads*)

Fig. 10 LS of apical region of a *K. bellardi* ectomycorrhiza showing a compact mantle (*), radially enlarged epidermal cells (*E*) and surface view (*arrowhead*) of Hartig net

Fig. 11 LS of *K. bellardii* ectomycorrhiza showing a compact mantle (*), radially enlarged epidermal cells (*E*) and well-developed Hartig net (*arrowheads*)

Fig. 12 Fluorescence microscopy of acriflavine-stained *K. bellardii* mycorrhiza. The mantle (*) and Hartig net hyphae (*arrowheads*) have stained positively

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