The fungal sheath of ectomycorrhizal pine roots: an apoplastic barrier for the entry of calcium, magnesium, and potassium into the root cortex?

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Abstract

The apoplastic permeability of the fungal sheath of two different ectomycorrhizal associations of Pinus sylvestris L. was analysed by laser microprobe mass analysis (LAMMA) and energy-dispersive X-ray spectroscopy (EDXS) after stable isotope labelling with ²⁵Mg, ⁴¹K and ⁴⁴Ca. Entry of ²⁵Mg and ⁴⁴Ca into the outer cortical apoplast of non-mycorrhizal roots was detected after 4 min of labelling. After a longer exposure time the endodermis with its Casparian band acted as an efficient apoplastic diffusion barrier for the radial movement of ²⁵Mg and ⁴⁴Ca into the stele. A fraction of approximately one-third of the apoplastic cations of the root cortex could not be exchanged against the external label even after longer exposure times. The ectomycorrhizal sheath of the two fungal species used, Pisolithus tinctorius (Pers.) Coker & Couch and Suillus bovinus (L. ex Fr.) Kuntze, does not completely inhibit the apoplastic movement of ions into the mycorrhizal root cortex, but retarded the penetration of isotopes into the cortical apoplast. In roots inoculated with S. bovinus, a clear labelling of the cortical apoplast could first be detected after 24 h of exposure to the stable isotope solution. At this time the labelling of the cortical apoplast in these mycorrhizal roots was higher than those of non-mycorrhizal roots and, with EDXS, changes in the element composition of the apoplast were detected. The results indicated that possibly hydrophobins localized in the fungal cell wall might be involved in the increased hydrophobicity of mycorrhizal roots and the lower permeability of the ectomycorrhizal sheath.

Key words: Apoplastic movement, ectomycorrhiza, endodermis, LAMMA, Pinus sylvestris.

Introduction

Taylor and Peterson (2000) showed that in root systems of field-grown Pinus banksiana Lamb. the dominant contributors to root length are the condensed tannin zone (72% of total) and the cork zone (10% of total). Neither the tannin zone nor the cork zone contributes significantly to the nutrient acquisition of the plant (Peterson et al., 1999). The regions of the highest ion uptake capacity, however, the non-mycorrhizal white zone (2% of total) or the ectomycorrhizas (16% of total) represent only a small fraction of the total root length (Taylor and Peterson, 2000).

An ectomycorrhizal root is characterized by two prominent structures, a fungal sheath completely surrounding the mycorrhizal root and the Hartig net, an intercellular network of hyphae within the root cortex, where the main exchange processes between the symbiotic partners are localized. Since there is no direct symplastic continuity between both partners, nutrients must pass the interfacial apoplast before they can be absorbed (Peterson and Bonfante, 1994). If the fungal mantle is impermeable to nutrient ions, the underlying root tissue would be isolated from the soil solution and an enclosed shared apoplastic nutrient exchange compartment will be created in which the conditions (e.g. pH, ion composition) can be controlled by both symbiotic partners efficiently. However, the presence of a barrier to apoplastic transport in the mantle would necessitate the uptake of nutrients from the soil.

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solution into the fungal symplast and, therefore, the mycorrhizal root would be completely dependent on the nutrient translocation by the fungal symbiont.

In general, a mycorrhizal infection can enhance nutrient uptake and thereby plant growth by (1) an increase of the absorbing surface area, (2) the mobilization of sparingly available nutrients or (3) an increased excretion of chelating compounds or exoenzymes. This positive effect of a mycorrhizal infection is well established for the macronutrients phosphate and nitrogen and some trace elements such as Cu and Zn (Marschner and Dell, 1994). However, knowledge of the effect of mycorrhizal infection on the uptake of nutrients such as K, Mg and Ca is limited and not consistent. For example, a reduction of the Ca tissue concentration in the root of ectomycorrhizal plants was shown by Lamhamedi et al. (1992). By contrast, by experiments with 45Ca or 25Mg a certain capacity of the external hyphae of VA mycorrhizal or ectomycorrhizal associations for the uptake and transport of these elements to the host root has been demonstrated (Rhodes and Gerdemann, 1978; Jentschke et al., 2000, 2001).

The permeability of an ectomycorrhizal fungal sheath has been examined by several authors using fluorochromes as apoplastic tracers, but the results were controversial. Ashford and coworkers (1988, 1989), for example, showed that the mantle of *Pisonia* and *Eucalyptus pilularis* Smith/ *P. tinctiorius* ectomycorrhizas is impermeable to the fluorescent dye cellufluor. However, it can be assumed, that the effect of an ectomycorrhizal sheath on apoplastic movement may differ with the fungal species, structure and properties of the mantle and possibly also with the growth conditions of the mycorrhizal root tips. Vesk et al. (2000) reported, after experiments with the fluorescent dye 8-hydroxyppyrene-1,3,6-trisulphonate (PTS), that the permeability of the fungal sheath of *Eucalyptus pilularis/P. tinctiorius* ectomycorrhizas grown on a wet agar surface differed from those grown in air.

In the experiments presented here, the stable isotopes 25Mg, 41K and 44Ca were used as apoplastic tracers. These isotopes represent the movement of ions across the fungal sheath better than fluorochromes, due to the higher size of dye molecules and different charge properties (Kuhn et al., 2000). The experiments presented here give specific information about the movement of ions via the fungal sheath into the root cortex in order to improve current knowledge about the contribution of these fungal symbionts to the nutrient acquisition of their host plants.

### Materials and methods

#### Culture of fungi and plants

The experiments reported here were carried out using isolates of *Suillus bovinus* received from RD Finlay, University of Lund, Sweden and *Pisolithus tinctiorius* from I Kottke, University of Tübingen, Germany. The fungi were cultured on modified Melin Norkrans (MMN) medium (Molina and Palmer, 1982) with 2% agar. Surface-sterilized seeds of *Pinus sylvestris* L. were sown in a sterilized sand/perlite mixture (3+1, v/v) and cultured for 3 months in a phytotron illuminated with 54 µmol m⁻² s⁻¹ with a day/night rhythm of 14/10 h at temperatures of 22/20 °C, respectively. For inoculation, the seedlings were transferred to 9 cm diameter Petri dishes with an opening for the stem, filled with sterilized perlite. The roots were separated from the perlite by charcoal filter paper (Schleicher & Schuell, Dassel, Germany). The inoculation of the plants was carried out by fungal inoculum placed in the Petri dishes close to young short roots.

#### Experimental design

For experiments the plants were carefully transferred into test tubes and preincubated in a nutrient solution containing 1.785 mM NH₄NO₃, 0.646 mM NH₄H₂PO₄, 0.017 mM FeCl₃, 3 µM MnCl₂, 0.3 µM ZnSO₄, and 1 mM KCl, MgSO₄ and CaCl₂. After 24 h the roots were labelled by substitution of the preincubation solution with a nutrient solution of chemically identical composition, but all of the Mg, K and Ca was replaced by enriched preparations of the stable isotopes 25Mg, 41K and 44Ca. Table 1 shows the natural isotope composition and the compositions of the stable isotope tracers as declared by the supplier. Control measurements by laser microprobe mass analysis showed that the percentage of the stable isotope labelling in the used nutrient solution was slightly lower than the supplier specification (Table 1).

After 4, 16 and 64 min, 24 h, 48 h and 72 h of exposure, the plants were removed from the labelling solution and parts of the root system with non-mycorrhizal and mycorrhizal fine roots were carefully blotted onto filter paper and immediately cryofixed by plunging in melting nitrogen or liquid propane. Samples of the labelling solution were frozen and used for control measurements with the laser microprobe mass analysis (Fig. 3A).

#### Sample preparation

The cryofixed samples were freeze-dried under low temperature and high vacuum conditions (CFD, Leica, Vienna, Austria). To prevent a recrystallization of water in the plant tissues, the freeze-drying was started at a temperature of −100 °C lasting for 7 d and continued for 2 d at −90 °C, −80 °C and −60 °C, respectively. Afterwards, the samples were slowly warmed to room temperature, followed by pressure-infiltration, according to a method described by Fritz.
Due to the fact that neither the material originally present in the plant before labelling nor the label are isotopically pure, but consist of a mixture of isotopes (i.e. Mg is composed of a natural mixture of $^{24}$Mg, $^{25}$Mg and $^{26}$Mg, Table 1) the data from the LAMMA measurements had to be corrected (Fain and Schröder, 1987). Especially after longer exposure times, the percentage of the label in the solution was reduced by isotopes released from the roots during the labelling. For that reason the identical apoplastic regions chosen for the LAMMA investigations were also analysed by energy-dispersive X-ray microanalysis.

The X-ray microanalytical studies were carried out under standardized conditions using a Philips EM 420 electron microscope provided with the DX4 system of EDAX. The spectra were collected between 0 and 20 keV with a Si(Li) X-ray detector with a thin beryllium window. For the measurements of 100 live seconds, an acceleration voltage of 120 kV, and an objective aperture of 70 μm were used. The calculated effective spot size ($D_{eff}$) under these conditions was 12 nm. Based on the interactions of the primary electron beam with the specimen, it has to be assumed that the real spot size was slightly larger. The element distribution was documented as a peak to background ratio (P/B) in order to diminish the effects of topographic irregularities of the sections during analysis. Due to the difficult standardization of the X-ray microanalytical technique, the P/B ratio was used as a semi-quantitative measurement of the element level in different apoplastic regions. Five X-ray microanalytical spectra were taken from every apoplastic area.

Statistical treatment

In the figures, mean values and SE (Fig. 3) or confidence intervals ($P < 0.05$, Figs 4, 5) as vertical bars are shown. The non-parametric u-test of Wilcoxon, Man and Whitney was used for statistical analysis of the data (Köhler et al., 1992).

Results

Structural preservation after cryofixation, freeze-drying and pressure infiltration

For the analysis of diffusible elements such as potassium and calcium, the commonly used technique for light- and electron microscopy with a hydrous chemical specimen preparation has to be replaced by techniques suitable to preserve the in vivo element distribution. In Fig. 2A–C the structural preservation of...
the roots after cryofixation, freeze-drying and pressure-infiltration of the material is shown. Due to the high water content, plant vacuoles in the root cortex collapsed and shrank as a result of ice-crystal growth during freezing and/or freeze-drying. The vacuolar content showed an electron-dense, heterogenous appearance. By contrast, the fungal cells of the sheath and the Hartig net were less susceptible to freezing artefacts. The apoplast of the fungal sheath, as well as the apoplast in distinct regions of the root, however, is clearly distinguishable.

The ectomycorrhizal sheath of the fungal symbionts surrounded the mycorrhizal root completely and consisted in both forms of two layers (Fig. 2B, C), an outer plectenchymatous layer with loosely arranged hyphae and an inner one with closely organized hyphae and a rather pseudoparenchymatous appearance. In the *P. tinctorius* ectomycorrhiza a tannin layer (Tl) is formed in inner parts of the hyphal sheath (Fig. 2B). Typically for gymnospermous roots, the Hartig net of both forms was multilayered, and penetrated the root cortex nearly up to the endodermis. In this region of the root, most endodermal cells have entered the second stage of development in which suberin lamellae are deposited on the inner wall surfaces and only some lamella-less passage cells remained in the primary stage with a Casparian band deposited in the anticlinal walls (Warmbrodt and Eschrich, 1985; Peterson et al., 1999).

### The stable isotope label detected by laser microprobe mass analysis

The $^{44}\text{Ca}$ and $^{25}\text{Mg}$-label was clearly detectable in outer regions of the non-mycorrhizal roots after 4 min (Fig. 3A, E). After this time, approximately 60% of the total Ca-content and 70% of the Mg-content of the outer cell wall of the epidermis originated from the external supplied label. By contrast, the labelling of the apoplast within the fungal sheath of the *S. bovinus* ectomycorrhiza was significantly lower. Compared to the divalent cations, the $^{41}\text{K}$ label was remarkably lower (Fig. 3I).

For all externally supplied isotopes, a clear and significant radial gradient from outer to inner parts of the root could be observed [$^{44}\text{Ca}$ and $^{25}\text{Mg}$ after 4 min (Fig. 3A and E), $^{41}\text{K}$ after 16 min (Fig. 3J)], indicating that no relevant relocation of the isotopes occurred during the preparation procedure.

After 16 min $^{44}\text{Ca}$ has penetrated the root apoplast of non-mycorrhizal roots up to the endodermis. 60% of the Ca-content in the outer root cortex and 25% of Ca in the inner cortical apoplast was exchanged against the external supplied label (Fig. 3B). The Ca and Mg content of the outer cell wall of the epidermis of non-mycorrhizal roots was nearly completely exchanged at this point in time (Fig. 3B, F). By contrast, in the apoplast within the sheath of *S. bovinus* and *P. tinctorius* ectomycorrhizas and in the interfacial apoplast of these roots only a low percentage of the Ca and Mg content was labelled.

After 64 min of exposure to the stable isotopes, the differences between non-mycorrhizal and mycorrhizal roots became smaller. Also in the ectomycorrhizal systems a clear labelling of the interfacial apoplast and of the outer cell walls of the endodermis was detectable (Fig. 3C, G). However, the entry of $^{44}\text{Ca}$ in ectomycorrhizal roots infected with *S. bovinus* was remarkably lower than in non-mycorrhizal roots or roots infected with *P. tinctorius* (Fig. 3C). The extension of the exposure time up to 64 min caused no visible change of the percentage label detected for $^{44}\text{Ca}$ or $^{25}\text{Mg}$ in the cortical apoplast of non-mycorrhizal roots. In non-mycorrhizal as well as in infected roots a clear and significant radial gradient of the labelling from the outer to the inner root parts was observed, and especially in the stele behind the Casparian band of the endodermis a remarkable decrease was detected.

In roots infected with *S. bovinus* a significant increase of the $^{44}\text{Ca}$, $^{25}\text{Mg}$ and $^{41}\text{K}$ label after 24 h was observed (Fig. 3J).
3D, H, L). By contrast, in non-mycorrhizal roots and roots infected with *P. tinctorius* the labelling of the distinct apoplastic regions remained nearly constant. Even though in *S. bovinus*-infected roots, nearly 80% of the Ca and Mg content within the root cortex was labelled, the labelling of the apoplast within the stele was significantly lower (Fig. 3D, H). A noticeable entry of the stable isotopes into the stele of pine roots was only detected for the monovalent cation $^{41}$K. After 24 h in the stele of non-mycorrhizal roots 25% of the apoplastic K content was labelled, whereas in the stele of mycorrhizal roots only low $^{41}$K contents were observed. The entry of $^{41}$K into the root cortex of pine roots was significantly reduced by an ectomycorrhizal infection with *P. tinctorius*.

A significant $^{25}$Mg and $^{44}$Ca labelling of the apoplast within the stele of non-mycorrhizal roots was first observed after 48 h (not shown). After 2 d of exposure to the labelling solution 23% or 28% of the Mg or Ca content, respectively, originated from the labelling solution. An extension of the exposure time up to 72 h led to a further increase of the $^{25}$Mg label (46%) within the stele.

**Element distribution determined by energy-dispersive X-ray microanalysis**

To get additional information about the radial distribution of the elements and to examine the effect of an ectomycorrhizal infection on the element levels, the same samples were also analysed with energy dispersive X-ray microanalysis.
X-ray microanalysis. The elements Ca and K were not homogeneously distributed across the non-mycorrhizal root cross-sections, but showed a characteristic radial distribution. The apoplastic Ca levels of the outer cell wall of the epidermis was significantly higher than in other parts of the root (Fig. 4A), whereas K clearly accumulated in the apoplast of the stele (Fig. 4D). In non-mycorrhizal roots neither for Ca nor for K was a clear effect of different exposure times on the apoplastic levels observed. This might be due to the fact, that all roots were preincubated...
for 24 h in a non-labelled, chemically identical nutrient solution before labelling. After this time an exchange equilibrium with the outer solution was achieved and only a time-dependent exchange, for example, of $^{40}\text{Ca}$ against $^{44}\text{Ca}$ could be observed, which was not detectable by EDXS.

By contrast to non-mycorrhizal roots, in roots inoculated with *P. tinctorius* $^{40}\text{Ca}$ was nearly homogeneously distributed (Fig. 4B). In particular, the Ca levels in the fungal sheath of both ectomycorrhizal associations were, compared to the levels in the epidermal apoplast of non-mycorrhizal roots, significantly lower. An ectomycorrhizal infection with *S. bovinus*, on the other hand, led to an accumulation of Ca in the interfacial apoplast between both symbionts (Fig. 4C). An extension of the exposure time led, in these roots, to a significant increase of the apoplastic Ca contents in the fungal sheath and the interfacial apoplast.

In both non-mycorrhizal and mycorrhizal roots a characteristic radial distribution of K with an increase of the apoplastic levels within the stele was found (Fig. 4D–F). Compared to the outer cortical apoplast of non-mycorrhizal roots, however, high K levels in the interfacial apoplast of mycorrhizal roots were also detected (Fig. 4E, F). By contrast to non-mycorrhizal roots and roots infected with *P. tinctorius*, where no time-dependent effect on the K levels in the apoplast was observed, in roots inoculated with *S. bovinus* a reduction of the apoplastic K levels up to the endodermis was found. By contrast, the K levels within the stele remained constant (Fig. 4F).

By contrast to Ca and K, Mg was nearly homogeneously distributed in non-mycorrhizal and mycorrhizal roots and no clear effect of the exposure time on the Mg levels in the apoplastic areas was found. Only in roots inoculated with *S. bovinus* was a slight decrease of the apoplastic Mg levels after long exposure times detected (not shown).

### The distribution of P and S in non-mycorrhizal and mycorrhizal roots

As observed for K, P and S were also accumulated within the central cylinder of non-mycorrhizal roots (Fig. 5A, D). In particular, the content of these elements was very low in the outer tangential cell wall of the epidermis. Compared to the apoplast of the endodermis, an increase of the P contents within the stele was also found in mycorrhizal roots (Fig. 5B, C). However, both ectomycorrhizal associations showed high P levels in their cell walls in sheath and Hartig net. After 24 h of exposure to the labelling solution in roots inoculated with *S. bovinus* a decrease of the P contents in the ectomycorrhizal sheath and in the interfacial apoplast was observed (Fig. 5C).

In roots inoculated with *P. tinctorius* a remarkable increase of the apoplastic S levels in the stele was found (Fig. 5E), whereas in roots infected with *S. bovinus* no accumulation of S in the central cylinder was detectable (Fig. 5F). An infection of the root with *P. tinctorius* led to significant higher S contents within the stele (Fig. 5E).

By contrast to non-mycorrhizal roots and roots inoculated with *P. tinctorius*, in mycorrhizas of *S. bovinus* a time-dependent effect was found on the element contents within the apoplast. In these roots an extension of the exposure time to the isotope solution caused an increase of the Ca contents in the interfacial apoplast and a significant decrease of the K and P contents within the sheath and the interfacial apoplast. In Fig. 6 this effect on the element composition is shown by X-ray element maps of the Hartig net area of *S. bovinus* ectomycorrhizas. After 16 min of exposure to the labelling solution, a high Ca content was detectable in the interfacial apoplast (Fig. 6B). High K and P levels were also found in this area, but these elements were also localized within the hyphae of the Hartig net, in the cytoplasm, in small granules, and in precipitated parts within the fungal vacuole (Fig. 6C, D). By contrast, after 24 h the Ca contents within the interfacial apoplast increased (Fig. 6F), whereas K and P were completely removed (Fig. 6G, H).

### Discussion

#### Specimen preparation for element analysis

The techniques used in these investigations, LAMMA and EDXS, allow measurements of stable isotopes and elements, respectively, at the inter- and intracellular level. However, this application requires a sample preparation technique, which perserve the *in vivo* element distribution. As expected, in non-mycorrhizal roots for all tested isotopes after short-time exposure a typical radial gradient with high exchange percentages in the outer parts and with lower percentages in the inner parts of the roots was detectable. The measured gradients give evidence that the element distribution was not significantly affected by the specimen preparation (cryofixation followed by freeze-drying and pressure infiltration) chosen for these experiments. Interestingly, this gradient was also found for the diffusible element $^{41}\text{K}$. In comparable experiments of Kuhn et al. (2000), who also used $^{41}\text{K}$ as a tracer in shock-frozen and cryosubstituted material, a gradient was not found and loss and redistribution of $^{41}\text{K}$ during the cryosubstitution was assumed.

#### The entry of stable isotopes into non-mycorrhizal roots

Solutes reaching the surface of a nutrient-absorbing root can move radially across the apoplast driven by diffusion and solvent-drag with the water or can be transferred symplastically, where the movement is rate-limited by passive or active permeation across the plasma membrane (Steudle and Peterson, 1998). Based on the observations that (1) the movement of Ca within the plant is much less...
energy- and temperature-dependent (Kirkby and Pilbeam, 1984; Kuhn et al., 2000), (2) the concentration of Ca in the cytoplasm is normally very low and is regulated by the activity of membrane-bound transporters (Evans et al., 1991), and (3) the uptake and radial movement of Ca is closely correlated with the differentiation of the endodermis (Häussling et al., 1988), it was supposed that Ca moves mainly apoplastically across the root. Mg has been reported to behave in the same way (Kuhn et al., 2000).

In the experiments presented here, after 16 min a clear $^{44}\text{Ca}$ and $^{25}\text{Mg}$-labelling of the apoplast of non-mycorrhizal roots up to the endodermis was observed and the Ca

Fig. 6. Part of the Hartig net in cryofixed, freeze-dried roots inoculated with $S.\ bovinus$ after 16 min (A–D) and 24 h (E–H) of exposure to the labelling solution. STEM image (A, E, scale bar, 1 μm), X-ray map of Ca (B, F), X-ray map of K (C, G), X-ray map of P (D, H). Abbreviations: CV, cortex vacuole; HN, Hartig net; IN, interfacial apoplast; P, polyphosphate granules in hyphae of the Hartig net (in C and D marked by arrows).
and Mg contents of the outer epidermal tangential wall were nearly completely exchanged against the external label. The X-ray microanalytical data indicate that the apoplast of these cells possess a large number of cation-binding sites, and that Ca is accumulated at the cell walls of the epidermis. The accumulation of Ca in outer regions of pine roots and especially of the epidermis was also shown by Bücking and Heyser (2000).

After 64 min of labelling, only at the outer cell wall of the endodermis was an increase of the percentage of the stable isotope label found. An extension of the exposure time up to 24 h caused no visible change of the labelling percentages of different apoplastic regions of the root and the gradient between epidermal and cortical apoplast on one side and between cortical apoplast and stele on the other side remained unchanged. Even after an exposure of 72 h to the labelling solution at least one-third of the apoplastic Mg and Ca content in the root cortex can not be exchanged against the externally supplied label. This is in contrast to Kuhn et al. (2000), who examined the entry kinetics of Ca and Mg into the cortex of spruce roots and reported that in the root cortex almost 100% of the cell wall Ca can easily be exchanged against an external 44Ca label.

The present investigations showed that the endodermis in non-mycorrhizal and mycorrhizal pine roots acts as an efficient apoplastic diffusion barrier, limiting the apoplastic movement of the divalent cations Ca and Mg. After long-time exposure to the stable isotopes 25Mg and 44Ca, only a low labelling of the apoplast within the stele was observed. The radial movement of Ca is closely correlated to the endodermal differentiation (Häussling et al., 1988) and it was shown that in non-mycorrhizal and mycorrhizal fine roots of pine the development of the Casparian band and the differentiation of the primary stage of the endodermis starts close to the root tip (Behrmann, 1995). This early differentiation of the endodermis in fine roots of pine might be an explanation for the low labelling rates found in this experiment for the divalent cations within the stele after long-time exposure. By contrast, Kuhn et al. (1995, 2000) found in mycorrhizal spruce roots, even after 2 h of exposure a significant labelling of the apoplast within the stele. The sections for the present investigations were made in a developmental stage of the root, where the first xylem elements were differentiated and most of the endodermal cells had reached the second stage of development in which suberin lamellae are deposited on all inner wall surfaces. The development of suberin lamellae and thick, secondary walls in the third stage of the endodermal differentiation does not generally prevent ion movement through the symplast. Plasmodesmata connecting the endodermis with the outer cortex and pericycle remain intact during the deposition of suberin lamellae or secondary cell wall material. It was assumed, however, that suberin lamellae, which tend to be impermeable to ions, would prevent Ca from contacting the plasma membranes in non-passage cells (Peterson and Enstone, 1996). This would considerably reduce the endodermal membrane surface area in short roots of pine suitable for a symplastic radial movement of Ca and Mg into the stele and might explain the very low exchange percentages observed in this compartment.

By contrast to the divalent cations 25Mg and 44Ca, a relatively low 44K label in the apoplast of non-mycorrhizal pine roots was found. The low labelling of the apoplast, in this case, might be due to an exchange with the symplastic ion pool. By contrast to Ca and Mg, K is taken up selectively, transported symplastically and the uptake rates along the roots are not related to changes in endodermal wall structure (Häussling et al., 1988). It was shown by several authors that the uptake of K into the symplast is localized in peripheral root tissues (MiströšKubicá, 1996). The X-ray microanalytical results showed clearly that K was mainly accumulated in the stele, whereas the contents, especially of the cortical apoplast, was relatively low. An accumulation of K in different cell compartments of the stele was described by several authors in herbaceous as well as in woody plant species (Läuchli et al., 1971; Bücking and Heyser, 2000). It was proposed that an increase in the osmotic potential of the stelar cells related to the K gradient may facilitate the movement of water across the root (Strasburger et al., 1998).

**The apoplastic movement of ions in ectomycorrhizal roots**

The apoplastic movement of ions in ectomycorrhizal roots across the fungal sheath has already been examined by several authors using fluorochromes as apoplastic tracers, but the results were controversial. For example, it was shown that the fungal sheath of *P. tinctorius* associated with different plant species prevented an apoplastic movement of the fluorescent dye cellulfluor (Ashford et al., 1988, 1989). By contrast, Behrmann and Heyser (1992) reported that there is a potential pathway for the fluorescent dye sulphorhodamine G and the heavy metal lanthanum across the fungal sheath of *S. bovinus* ectomycorrhizas. Vesk et al. (2000) found, that besides species-dependent differences, growth conditions also affected the permeability of the fungal sheath. Mycorrhizas in contact with a wet agar surface remained permeable to a fluorescent dye while those developed in air excluded the fluorochrome. However, movement and diffusivity of large uncharged or negatively charged dyes might be quite different from that of hydrated inorganic ions and especially of ions which are potential nutrients for the fungal symbiotic partner.

Therefore, stable isotopes are a useful tool to get more information on this subject, since they are chemically identical tracers for nutrients which have to pass the fungal sheath in ectomycorrhizal associations before they can be absorbed by the root cortex (Kuhn et al., 2000). In these
experiments the movement of the stable isotopes $^{25}$Mg, $^{41}$K and $^{44}$Ca across the fungal sheath of two ectomycorrhizal associations were tested and the results showed clearly that the fungal sheath of these ectomycorrhizas allowed a radial apoplastic movement of these elements and does not seal the root cortex completely. However, the labelling of the wall surfaces in mycorrhizal roots and particularly of roots inoculated with S. bovinus are retarded compared to non-mycorrhizal roots. Possibly this effect is due to the high water repellency of the ectomycorrhizal sheath. Both ectomycorrhizal fungi are known to belong to hydrophobic fungal species (Unestam, 1991). The water-repellency of fungal sheaths is caused by hydrophobins which are small, secreted, moderately hydrophobic proteins with a conserved spacing of eight cysteine residues (Kershaw and Talbot, 1998; Wessels, 1999). Interestingly, in these investigations an apoplastic accumulation of S in the fungal sheath and interfacial apoplast of P. tinctorius ectomycorrhizas was detected. Due to their high cysteine content hydrophobins are known to be S-rich proteins and this might be an indication that these hydrophobins are involved in the higher diffusion barrier of these mycorrhizal roots. In the early stages of ectomycorrhizal differentiation between Eucalyptus globulus and P. tinctorius an upregulation of hydrophin encoding genes was determined (Tagu et al., 1996). Unestam and Sun (1995) assumed that if the hyphal walls contain and are cemented together by hydrophobins, neither the interhyphal space nor the cell walls are likely to participate in apoplastic translocation in cords and hyphae.

However, after 24 h of exposure to the stable isotopes, a significant labelling of the cortical apoplast was also found in ectomycorrhizas, and in the apoplast of S. bovinus inoculated roots remarkable changes can be observed. Based on the X-ray microanalytical results, it can be assumed that the entry of the divalent cation Ca into the cortical apoplast of these roots and the occupation of cation-binding sites removed K. By contrast, neither in non-mycorrhizal nor in P. tinctorius infected roots was a time-dependent effect on the ion composition of the apoplast observed. A possible explanation for the loss of K from the apoplast of the S. bovinus-infected roots might be that, in this case, the preincubation of the roots for 24 h in a non-labelled nutrient solution was too short to get an exchange equilibrium and to balance the ion conditions within the apoplast. This would emphasize the water repellency of the ectomycorrhizal sheath of S. bovinus. Possibly the long incubation time (24 h preincubation and 24 h labelling) caused a significant loss of hydrophobins localized in the apoplast, and therefore increased the permeability of the fungal sheath. Vesk et al. (2000) also reported, that the differences in the permeability of mycorrhizas for a fluorescent dye grown on a wet agar surface and those developed in air are possibly due to a loss of hydrophobins from the cell wall surface by diffusion into the medium. The possible loss of hydrophobins from the cell wall surfaces of the S. bovinus ectomycorrhiza is also indicated by the X-ray microanalytical results. After 24 h of exposure, lower apoplastic S contents were found in the sheath and the interfacial apoplast. After 24 h in the interfacial apoplast and the outer tangential cell wall of the endodermis of S. bovinus-infected roots, higher exchange percentages than in non-mycorrhizal roots or P. tinctorius ectomycorrhizas were found. This effect might be due to an alteration of the cell wall composition of these mycorrhizal roots leading to a higher exchangeability of the cations in the cortical apoplast. Modifications of the host cell wall as a consequence of an infection of pine roots by S. bovinus were reported also by Duddridge and Read (1984).

The LAMMA experiments presented here clearly showed, that the ectomycorrhizal sheath is not totally impermeable as formerly proposed, but that ions can move across and that ectomycorrhizal roots are not completely cut off from an external nutrient supply. However, the possibility of an apoplastic nutrient transport across the fungal sheath in ectomycorrhizal roots does not mean that, under normal soil conditions where depletion zones around ectomycorrhizal roots occur and the main nutrient acquisition of mycorrhizal systems takes place at the extraradical mycelium, the fungal symptom does not compete with its host plant for limited nutrient resources. In these experiments the $^{41}$K entry within the root cortex was significantly reduced by P. tinctorius, possibly by an uptake of K into the fungal sympat. In this case the supply of the host plant with this nutrient depends on the ion concentration within the fungal sympat of the Hartig net and the translocation rate of these ions across the fungal plasma membrane into the interfacial apoplast.

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References


