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Xerocomus badius – Picea abies, an ectomycorrhiza of high activity and element storage capacity in acidic soil

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Abstract Mycorrhizas were collected from three Norway spruce (*Picea abies*) stands in southwest Germany, sorted on the morphotype level and analysed by fluorescein diacetate vital fluorescence staining and the accumulation of elements using inductively coupled plasma-atomic emission spectrometry (ICP-AES) and electron energy-loss spectroscopy (EELS). *Xerocomus badius – Picea abies* mycorrhizas showed a higher frequency of active hyphal sheaths and a higher potential to store nitrogen, phosphorus, potassium, magnesium, iron and zinc than other mycorrhizal types. Phosphorus and nitrogen were localized by EELS in vacuolar bodies which occurred consistently in the sheath of *X. badius* mycorrhizas. The results indicate that *X. badius* is well adapted to acidic stands and that its mycorrhizas are very efficient in uptake and storage of macronutrients.

Key words Ectomycorrhizas · EELS · Element accumulation · Hyphal sheath · ICP-AES · FDA hydrolysing activity

Supplementary material Figures 1–5 have been deposited in electronic form and can be obtained from: http://link.springer.de/journals/myco/

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Introduction

Although it is known that northern hemisphere forest trees are simultaneously associated with a great variety of ectomycorrhizal fungi, very little information is available on the supposedly varied benefits to nutrient uptake conferred by the diverse mycorrhizal types on mature trees in natural stands. Experimental data clearly indicate differences in the efficiencies of fungi. Experimental inoculation of *Eucalyptus* spp. seedlings by diverse ectomycorrhizal fungi produced different growth responses connected to uptake rates of phosphorus (Burgess et al. 1993). *Laccaria laccata* (Scop. ex Fr.) Bk & Br. was more efficient than *Thelephora terrestris* Pers. ex Fr. in increasing the growth, photosynthesis and water-use efficieny of *Pseudotsuga menziesii* (Mirb.) Franco seedlings at low P levels in the substrate. This effect was correlated with improvement of phosphorus nutrition and growth of external mycelium (Guehl and Garbaye 1990). Mycelial acid phosphatase activity differed between common ectomycorrhizal fungi (Ho and Zak 1979; Dighton 1983), and uptake rates of ammonium, nitrate, K, Ca and Mg were also found to differ between mycelia of four mycorrhizal species grown in liquid culture (Mention and Plassard 1983).

In mature forests, attempts to measure element uptake rates or specific enzymatic activities of different mycorrhizal types are confronted with severe difficulties. The main problem is the sampling of well-defined material in sufficient amounts. The total amounts of elements and the overall metabolic activity of mycorrhizas can, however, be determined from preliminary knowledge on the efficiency of different mycorrhizal types. Göbl (1967) investigated the total amounts of the elements P, K Ca and Mg in six not-identified mycorrhizal types of *Pinus cembra* L. P and K concentration levels were considerably higher in a white tuberculate mycorrhiza than in the other types and black mycorrhizas had the lowest amounts of elements.

Fungi store elements in the vacuoles (Klionsky et al. 1990) but also fix them to pigments or chelated prod-

ucts (Gadd 1994). There is good evidence that P is stored as polyphosphate in vacuoles (Cairney et al. 1988; Klionsky et al. 1990; Martin 1991; Ashford et al. 1994). Investigations carried out by electron microscopy and micro-analysis revealed vacuolar inclusions of high phosphorus content in mycelia of ectomycorrhizal fungi and in the hyphal sheath of mycorrhizas (Ashford et al. 1986; Grellier et al. 1989; Väre 1990; Kottke and Martin 1994). Orlovich and Ashford (1993) declared the polyphosphate granules to be artefacts of dehydration during the preparation processes, although they did not doubt storage of polyphosphate in the fungal vacuole (Ashford et al. 1994). These authors suggested the transportation of polyphosphates by peristalsis along tubular vacuoles in a soluble state. However, granules can also be observed moving by peristalsis in living hyphae (Bücking et al. 1997; I. Kottke unpublished work). The presence or absence of P-containing granules in mycelia may depend on the age of the culture, the amount of phosphorus applied and the accompanying cations. In mycelia of *Paxillus involutus* (Batsch) Fr. at the oldest phase of culture, a small pool of polyphosphates was found by P^{31}NMR spectroscopy, but this was missing in rapidly growing cultures where the main pool was orthophosphate (Grellier et al. 1989). Interestingly, prominent polyphosphate peaks were obtained by studying the mycorrhizas of the same fungus (*Paxillus involutus* with *Betula pendula* Roth).

The results indicate differences in metabolism of the free-living and the symbiotic stages of mycelia (Grellier et al. 1989). Diverse cations have been detected associated with the P-containing granules in mycelia of ectomycorrhizal fungi and the hyphal sheath (Strullu et al. 1982; Ashford et al. 1986; Turnau et al. 1993, 1994; Bücking and Heyser 1997). The composition of cations was partly influenced by the preparation technique. While Ca was found as the main counter-ion of polyphosphate in chemically fixed material, K was the associated ion in freeze-dried material (Orlovich and Ashford 1993; Bücking and Heyser 1997). Amounts of P and K in the vacuoles were lowered during chemical fixation (Young et al. 1993; Bücking et al. 1997).

There is evidence from physiological analyses that amino acids are stored in the fungal vacuoles and may in part be bound to polyphosphate (Klionsky et al. 1990). Interestingly, nitrogen was detected in the P-containing vacuolar inclusions by means of electron energy-loss (EEL) spectroscopy in the hyphal sheaths of *Cenococcum geophilum–Pinus sylvestris* mycorrhizas (Kottke et al. 1995a). The amounts of stored nitrogen in the vacuolar granules were correlated with the amount of N fertilizer applied. Fungal vacuoles are, without doubt, a compartment of storage for nutritive or detrimental elements (Klionsky et al. 1990). In a comparative investigation of freeze-dried and of chemically fixed hyphae of *Paxillus involutus* and *Xerocomus badius,* P was detected by X-ray microanalysis and N was found by EEL spectroscopy in the vacuolar bodies (Bücking et al. 1997).

In ectomycorrhizas, the hyphal sheath is generally looked upon as the compartment for the storage of elements (Harley and McCready 1952; Sihanonth and Todd 1977). The amounts of stored elements do not by themselves reflect nutrient uptake rates and, therefore, the metabolic activity of ectomycorrhizas should also be measured. A simple method to determine overall activity of plant and fungal cells is vital staining by fluorescein diacetate (FDA) (Ziegler et al. 1975; Larkin 1976), where the stain is hydrolysed by unspecific cytoplasmic esterases into fluorescein and acetate. The fluorescein molecule accumulates in the cytosol giving bright green fluorescence under UV light. Söderström (1977, 1979) investigated mycelia of 50 different fungal species from pure culture and soil and found good correlations between growth rates, respiration rates and fluorescence intensities. All mycelia were permeable and gave the same bright fluorescence when in an active state. Comparable results on infiltration were obtained by staining hand-sectioned mycorrhizas from culture (Downes et al. 1992) or collected from forest stands (Ritter et al. 1986). All the fungi displayed a bright fluorescence when in an active state. Both investigations found that FDA staining reflected cell activity and cell death as seen in morphological and ultrastructural studies (Ritter et al. 1986; Downes et al. 1992). Comparison of photosynthetic oxygen evolution and FDA fluorescence of single protoplasts revealed that integrity as measured by FDA was nearly unchanged with incubation time, while O_2 evolution decreased, indicating different speeds of deterioration of physiological and plasma membrane integrity (Hampp et al. 1986). Despite the restrictions of vital staining (Bornman et al. 1982), in field studies FDA staining may still serve as an appropriate indicator of overall viability.

Here we present data on amounts of stored elements and the FDA-hydrolysing activities of a number of mycorrhizal types collected in three Norway spruce [*Picea abies* (L.) Karst] plots. Special emphasis is placed on results obtained from the mycorrhizas formed by *Xerocomus badius* (Fr.) Kühn. ex Gilb which appeared as a dominating type in the acidic Norway spruce stands.

Material and methods

Sampling of mycorrhizas

Mycorrhizas were sampled in three mature Norway spruce stands in southwest Germany which had been extensively studied during tree-decline research. Two stands are situated in the southern Black Forest (Villingen and Schluchsee, ARINUS-project control plots; Haug and Feger 1990/91). The third stand is part of the Höglwald near Augsburg (Bavaria), where two plots had been treated experimentally by acid water or acid water and lime over a 6-year period (Kreutzer et al. 1991). The pH on all plots ranged between 3.2 (organic layer, moder humus) and 4.4 (30 cm depth, mineral soil).

Sampling of the Black Forest plots was carried out by digging fine-root systems from the organic layer and the humus-rich mineral soil within 2 m of the tree trunks. On the Höglwald plots, sampling was done using a 5-cm-diameter soil corer to a depth of

30 cm, taking 5 samples each time. The samples were stored at 4 °C up to 1 week. Previous investigations had shown that samples could be stored up to 2 weeks without loss of integrity (Ritter 1990). The mycorrhizas were selected by hand from the soil cores using a stereomicroscope. Active-looking mycorrhizas, indicated by a light apical meristem, a perfectly ensheathing hyphal mantle, and turgescent root cortical cells were selected from the fine-root systems sampled on the Black Forest plots. These were then identified and used for measurement of elemental contents. Only obviously dead mycorrhizas, which could no longer be identified, were eliminated from the mycorrhizal samples of the Höglwald plots. The rest were identified and used for evaluation of FDA-hydrolysing activity. Mycorrhizal types were identified on the fungal species or the morphotype level according to Agerer (1987–1996), Haug (1987), Haug and Pritsch (1992), and Gronbach (1988).

Measurements of total amounts of elements

Seventeen mycorrhizal types were obtained from the Villingen and the Schluchsee plots by monthly sampling from July until December 1989 in sufficient numbers to measure total amounts of elements. The required dry weight of 30–100 mg of each mycorrhizal type was equivalent to 500–1000 living tips. The following identified mycorrhizal types *Lactarius sp.–P. abies, Paxillus involutus–P. abies, Piloderma croceum–P. abies*, *Russula ochroleuca–P. abies, Tuber sp.–P. abies, Tylospora fibrillosa–P. abies, Xerocomus badius–P. abies* and the morphotypes *Cenococcum geophilum*, *Piceirhiza echinacea*, *Piceirhiza gelatinosa*, *Piceirhiza licheniformis*, *Piceirhiza rosa-nigrescens*, *Piceirhiza rosea, Piceirhiza viridis*, white type wS1, white type wS6, white type wV were included in the element measurements. For a detailed morphotype description, see Haug and Pritsch (1992).

Mycorrhizas were cleaned in deionized, cooled water using tweezers and a fine brush with the help of a stereomicroscope. The material was kept in water for a constant time of 1 h as a precaution against elements moving within the tissues or leaching out. Mycorrhizas were air dried and stored at room temperature. The material was then oven dried at 70 °C for 1 h and 35–120 mg of dried mycorrhiza was prepared for elemental analysis. The total amounts of P, K, Ca, Mg, and the microelements Fe, Zn, Mn and Al were measured by flame-spectrometry with an inductively coupled plasma-atomic emission spectrometer (ICP-AES; Perkin-Elmer ICP/5500B). Sufficient amounts of mycorrhizas for 2–4 replicate measurements were only available for 9 of the 17 types. The number of repetitions is in any case not sufficient for statistical analysis and only means and standard deviations are presented.

Evaluation of the activity of mycorrhizal sheaths

Mycorrhizas (2590 samples in total) collected in February, April, June, August and October 1990 and 1991 on the Höglwald plots were examined for overall activity of hyphal sheaths. The six dominating mycorrhizal types, *Tylospora fibrillosa–P. abies* (856 samples), *Russula ochroleuca–P. abies* (576 samples), *Xerocomus badius–P. abies* (288 samples), *Piceirhiza nigra* (680 samples), *Piceirhiza conspicua* (190 samples) are considered in the present study. The activity of the mycorrhizal sheaths was judged visually in terms of green fluorescence under UV light after FDA staining (Ziegler et al. 1975; Ritter et al. 1986; Hampp et al. 1986; Downes et al. 1992). Coloured photographs of several mycorrhizal types treated with FDA have been published previously (Ritter et al. 1986, see *R. ochroleuca–P. abies* Figs. 3, 15 and 17; *Piceirhiza nigra* Figs. 7, 8, 9) and are accessible as electronic supplementary material (http://science.springer.de).

Longitudinal hand-cut sections of about 0.5 mm thickness were incubated for 5 min in FDA (Sigma, 0.01 mg ml^{-1} in phosphate buffer pH 7.5) on a microscope slide. Sections were washed on the slide 3 times in a pure phosphate buffer (pH 7.5), absorbing the FDA with a strip of filter paper. Sections in the buffer solution were then covered by a cover slip and analysed immediately using fluorescence microscopy (UV-filter BP 365/FT 395 LP 397, HBO 100 W lamp). Visual diagnosis was completed within 1 min of exposure to the UV light. The percentage of mycorrhizas of each type displaying bright green fluorescence in the hyphal sheath was calculated, irrespective of the number of fluorescing cells. Thus, the data obtained present a rough evaluation of the FDA accumulative and hydrolysing activity of the cytosol of the hyphal sheath. Data obtained on other layers of the mycorrhizas are presented elsewhere (Qian et al. 1997). Despite the many mycorrhizas sectioned and investigated, no statistical analysis could be carried out due to the extreme variation in occurrence of mycorrhizal types within the plots and in the amounts of each type found on each sampling date. Therefore, the interpretation of the presented data relies on trends indicated by pooled samples.

Ultrastructural studies and EELS

Ultrastructure was studied in the mycorrhizal types *Xerocomus badius–P. abies, Russula ochroleuca–P. abies, Tylospora sp.–P. abies*, and *Piceirhiza nigra*. Active mycorrhizas as described above were fixed in 2.5% glutaraldehyde in HEPES buffer (pH 7), postfixed in 1% osmium tetroxide, and embedded in epoxy resin using acetone for dehydration (Kottke and Oberwinkler 1988). Semithin sections were stained by crystal violet, which produces a light red colour in the cytosol and a bright metachromatic contrast to what have been interpreted as polyphosphate bodies (Chilvers and Harley 1980). Sections of 70 nm thickness were deposited on Formvar-covered copper grids and stained by uranyl acetate and lead citrate. Samples were observed using a Zeiss TEM902.

X. badius mycorrhizas contained large amounts of osmiophilic vacuolar bodies. This material was selected for analysing the elemental composition of the vacuolar bodies using EELS. Sections of 30–40 nm thickness were deposited on copper grids immediately after cutting and analysed without further treatment by EELS using a Zeiss TEM902 (filter type Castaing-Ottensmeyer, serial energy-loss spectroscopy, EELS 13 software vers.1.3) (Kottke and Martin 1994; Kottke et al. 1995a). Vacuolar bodies (100 in total) in the mycorrhizal sheaths were examined for P, N and Al. Spectra were acquired at a magnification of 30 000 (N, P) or 85 000 (P, Al) depending on the diameter of the bodies. The advantages of the EELS method are the precise subcellular localization and the possibility to identify and measure the relative amounts of N, which is not possible by either ICP-AES or X-ray microanalysis. The disadvantages lie in the embedding procedure, the lack of reference data for quantification and the very low measurable volume (area diameter of 360 or 130 μm). No quantification of the elements was attempted and only the typical element spectra obtained are presented.

Results

The Norway spruce plots in the Höglwald and in the Black forest turned out to be similar in the diversity of dominating mycorrhizal types. *X. badius–P. abies* mycorrhizas ranged from 2.3% (untreated plots) to 14% (artificially acidified plots) of the dominating types (Haug et al. 1992; Taylor et al. submitted for publication). Frequency of *X. badius–P.abies* mycorrhizas increased after artificial acidification of the soil and this mycorrhiza almost disappeared after pure liming of the Höglwald plots. Thus, *X. badius* appears to be well adapted to acidic soils.

The total amounts of elements measured by ICP-AES in the various mycorrhizal types are presented in Fig. 1. *X. badius–P. abies* mycorrhizas exceeded all oth-

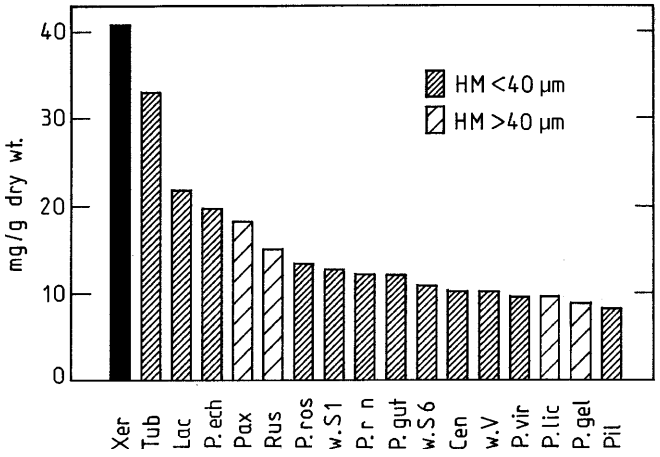

Fig. 1 Total amount of elements in the identified mycorrhizal types and morphotypes from the Villingen plots measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES). *X. badius* mycorrhizas hyphal sheath > 40 µm thick (*Xer Xerocomus badius, Tub Tuber puberulum*, *Lac Lactarius* sp., *P.ech Piceirhiza echinata*, *Pax Paxillus involutus*, *Rus Russula ochroleuca*, *P.ros. Piceirhiza rosea*, *w.S1* white type 1, *P rn Piceirhiza rosa-nigrescence*, *P.gut Tylospora fibrillosa*, *w.S6* white type 6, *Cen Cencoccum geophilum*, *wV white Villingen*, *P.vir Piceirhiza viridis*, *P.lic Piceirhiza licheniformis*, *P.gel Piceirhiza gelatinosa*, *Pil Piloderma croceum*, *HM* hyphal sheath thickness in µm

er mycorrhizal types with concentrations of about 40 mg g^{-1} dry wt. *T. puberulum–P. abies* was also an accumulator of elements (33 mg g^{-1} dry wt.), but at concentrations lower than in *X. badius*. The other 15 types contained about the same total amounts of elements (10–20 mg g^{-1} dry wt.). *X. badius–P. abies* mycorrhizas accumulated exceptionally high amounts of P and K (Fig. 2), but concentrations of Mg, Zn and Fe were also higher than in most other mycorrhizal types (Figs. 2, 3). Amounts of Ca, Mn and Al were surprisingly low in *X. badius–P. abies* mycorrhizas (Figs. 2, 3). Because of the low number of replicates (2–4, Table 1), statistical analysis for significance of differences was inappropriate. The variability in amount of elements between replicates (standard deviation as percentage of mean in Table 1) was below 30% for the majority of the elements, which is considered to be low for field material. Only Al was found to vary by more than 30%, most likely due to clay particles incorporated in the hyphal sheaths. In two mycorrhizal types (*Piceirhiza rosea*, *Russula ochroleuca–P. abies*), element content was also found to be highly variable. In the first case, this may be explained by differing amounts of soil nutrients at the two sampling points (not shown). In the case of *R. ochroleuca,* no explanation is so far possible. No close connection between the total amounts of elements and the thickness of the hyphal sheaths of the different mycorrhizal types was found (Fig. 1), but *X. badius* formed the thickest sheath (>40 µm) of all types investigated.

Analysis of the general FDA-hydrolysing activity of the five mycorrhizal types showed that *X. badius–P. abies* mycorrhizas had by far the highest percentage frequency of fully vital sheaths (19%, Table 2). While *Piceirhiza nigra* displayed about 5% actively fluorescing hyphal sheaths, values for the other three types were 1% or below (Table 2).

The ultrastructural analysis showed that cells of the hyphal sheath of *X. badius–P. abies* were alive throughout and contained cytoplasmic organelles (Figs. 4, 5). Light and electron microscopy revealed a high frequency of vacuolar metachromatic and osmiophilic bodies, respectively, occurring regularly in the outer layers of the hyphal sheaths (Fig. 4). Two types of osmiophilic

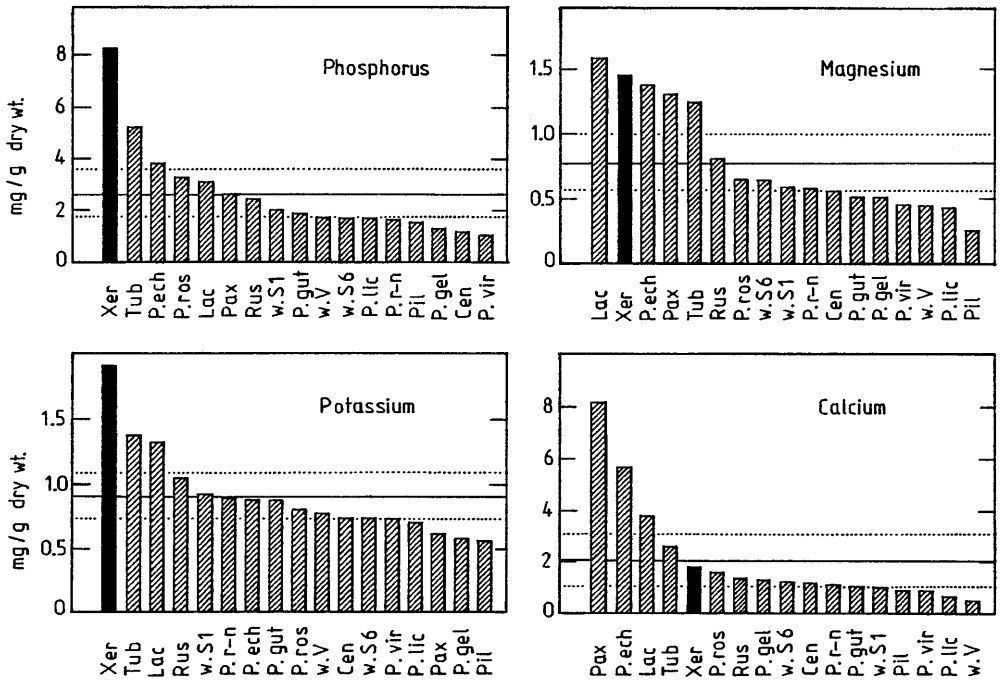

Fig. 2 Amounts of phosphorus, magnesium, potassium and calcium in the mycorrhizal types from the Villingen plots measured by ICP-AES. For abbreviations of mycorrhizal types see Fig. 1 (h*orizontal line* mean value of all types, *dotted lines* standard deviation)

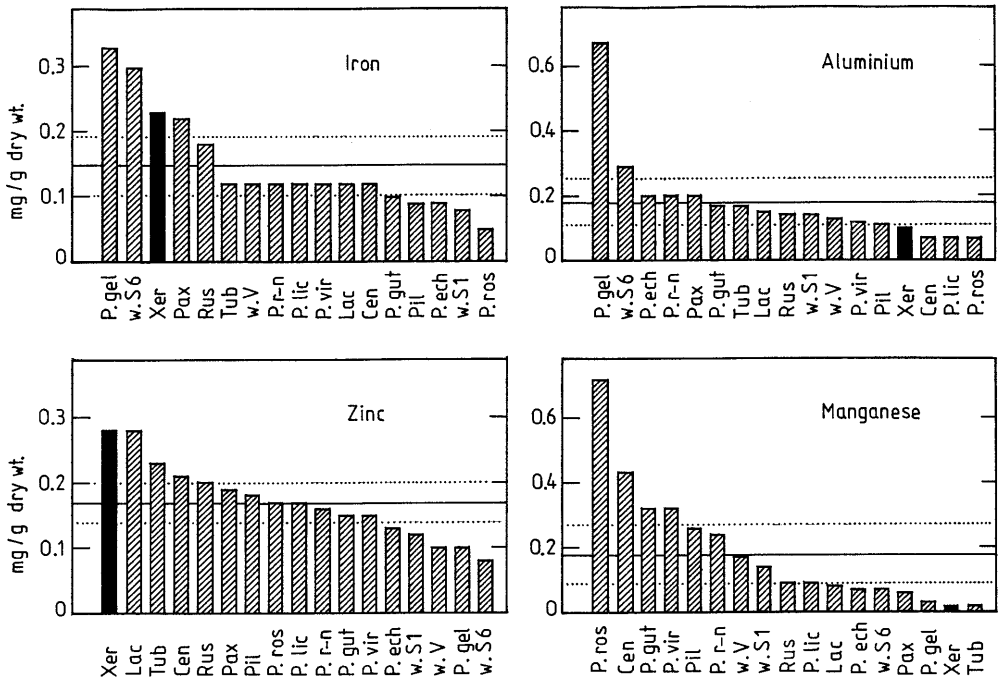

Fig. 3 Amounts of iron, aluminium, zinc and manganese in the mycorrhizal types from the Villingen plots measured by ICP-AES. For further explanation, see Figs. 1, 2

Table 1 Standard deviations in percentage of the means (variability coefficients) of the total amounts of elements measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES) in different mycorrhizal types (*Cen Cenococcum geophilum, Lac Lactarius sp., n* number of replicates, *P.gut Tylospora fibrillosa, P.lic Piceirhiza cheniformis, P.ros Piceirhiza rosea, Rus Russula ochroleuca, Tub Tuber puberulum, w.S6* white type 6, *Xer Xerocomus badius*)

	Xer n=3	Tub 2	P.ros 4	Lac 3	Rus 2	P.gut 4	w.S6 2	P.lic 3	Cen 3
P	11	0.2	44	10	55	21	5	7	15
K	8	7	11	7	27	10	2	8	12
Mg	13	6	43	16	53	11	3	7	30
Ca	20	6	49	4	38	20	27	7	27
Fe	22	4	14	25	33	33	13	5	22
Zn	26	26	53	25	3	34	7	24	33
Al	50	40	17	54	55	42	24	14	34
Mn	15	20	68	13	22	46	1	7	22

Table 2 Percentage frequency of mycorrhizas displaying bright green fluorescein diacetate fluorescence in hyphal sheats

Mycorrhiza	Number of replicates	Percentage mycorrhizas with active hyphal sheath
Tylospora fibrillosa–P. abies	856	0.69
Piceirhiza nigra	680	5.29
Piceirhiza conspicua	190	0.50
Russula ochroleuca–P. abies	576	1.62
Xerocomus badius–P. abies	288	18.93

bodies were detected in the vacuoles of *X. badius–P. abies* sheaths (Fig. 5). Large bodies with a diffuse border occurred in the expanded vacuoles, while small, more distinctly lined bodies were found predominantly in the small vacuoles (Fig. 5).

Vacuolar bodies were observed inconsistently in the other three mycorrhizal types, partly due to the less-active state of the hyphal sheaths. Typical pictures of the state of the hyphal sheaths of *Tylospora fibrillosa, Russula ochroleuca* mycorrhizas and *Piceirhiza nigra*, have been published previously (see Haug et al. 1986). In both mycorrhizal types, the outer layers of the hyphal sheaths consisted mainly of moribund hyphae (compare Fig. 3 of *R. ochroleuca*, Fig. 7 of *Tylospora fibrillosa* mycorrhizas and Fig. 4 of *Piceirhiza nigra* in Haug et al. 1986).

EELS revealed that the small, distinctly lined bodies contained P in high amounts and occasionally also Al, but N was absent (Fig. 6, left column). The large, more-diffuse bodies contained N in high amounts, but P was frequently at the limit of detection (Fig. 6, right column). Al was never detected in the large bodies (Fig. 6).

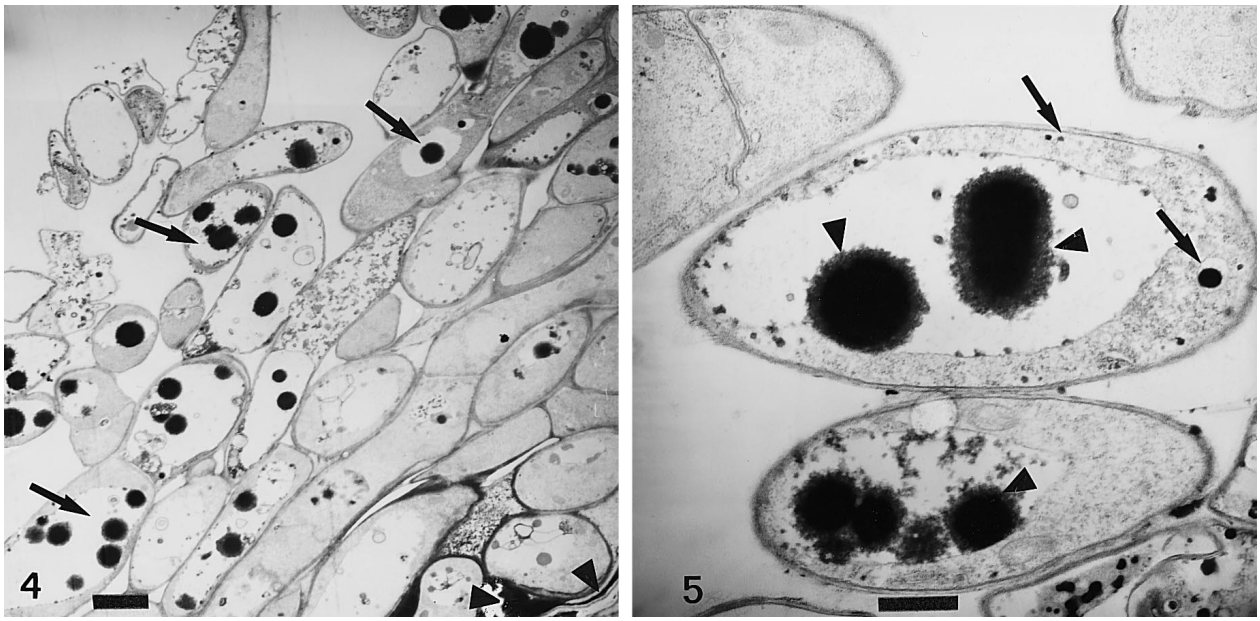

Fig. 4 Typical cellular state of the hyphal sheath of *Xerocomus badius* mycorrhizas. Living cells predominate throughout the sheath. The hyphae in the outer layer of the sheath contain osmiophilic bodies in enlarged vacuoles (*arrows*) (*arrowheads* root cortical cell); *bar* 2.5 μm

Fig. 5 Two kinds of bodies in the vacuoles are visible: large bodies are diffusely lined (*arrowheads*), small bodies are distinctly lined (*arrows*); bar 1 μm

Discussion

The studies carried out on three Norway spruce stands using various techniques to define functional aspects of ectomycorrhizas all illustrated exceptional activity and storage capacity of *X. badius* mycorrhizas. *X. badius* mycorrhizas are well adapted to acidic stands, becom-

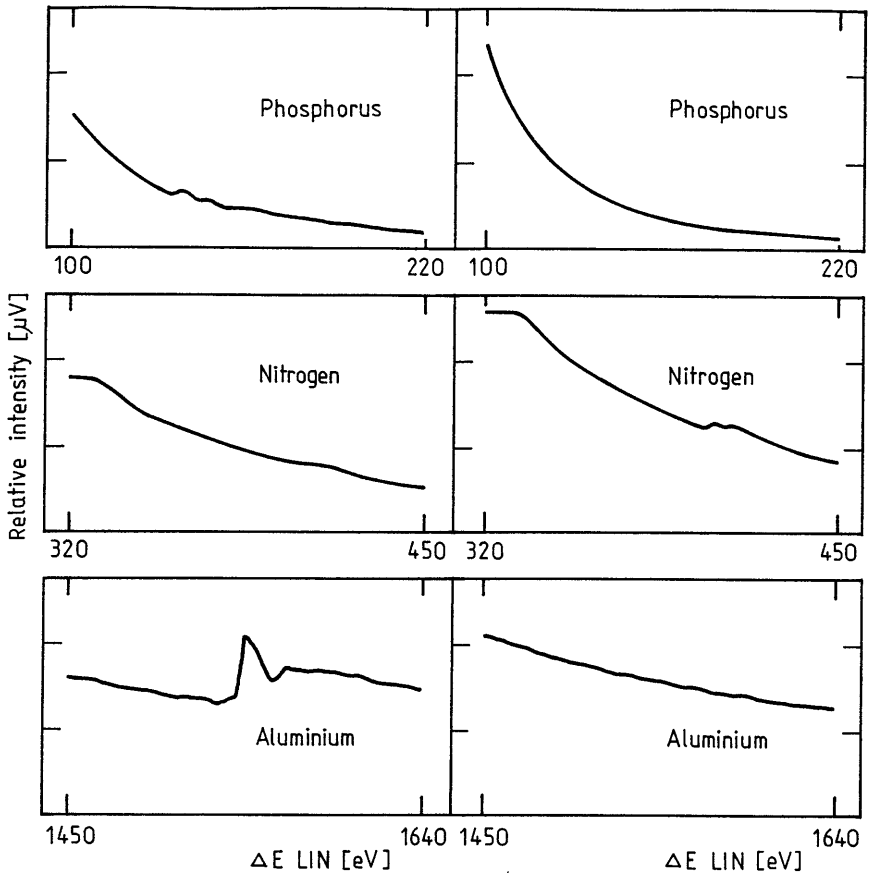

Fig. 6 Typical electron energy loss spectra obtained from the vacuolar bodies in the hyphal sheath of *Xerocomus badius* mycorrhizas. *Left*:results obtained from the small bodies, with prominent phosphorus peaks and occasionally aluminium, but no nitrogen. *Right*: results from the large bodies with prominent nitrogen peaks, but phosphorus at the detection limit, no aluminium

ing more frequent after artificial acidification, and a high percentage displayed bright FDA fluorescence in the hyphal sheaths. The high storage capacity appeared to be connected to both activity of the hyphal sheath and to the frequent occurrence of vacuolar bodies containing N and P. *X. badius* is thus a functionally important ectomycorrhizal fungus on acidic stands, where nutrient availability is normally low.

Although the intensity of FDA vital florescence was evaluated only subjectively, the method allowed screening of high numbers of mycorrhizas on the type level to determine the functioning of different tissues (Kottke et al. 1993). Detailed physiological methods need still larger amounts of material, which may be difficult to obtain from the field. Given that all mycorrhizal types displayed bright FDA fluorescence, at least in some replicates, there was no indication of different stain permeability of the sheaths. Similar results on staining of different ectomycorrhizal and soil-inhabiting fungi were reported by Söderström (1977, 1979). The results obtained by FDA fluorescence are supported by previous transmission electron microscope (TEM) studies in which mycorrhizas were cut into two and studied using both FDA fluorescence and TEM techniques (Ritter et al. 1986). We embedded active-looking mycorrhizas of each type and present the most frequently found active cellular state of *X. badius* mycorrhizas. In a previous publication, we showed typical states of the hyphal sheath of other mycorrhizal types: *Russula ochroleuca–Picea abies* (type 3); *Piceirhiza nigra* (type 4), *Tylospora fibrillosa–Picea abies* (type 7 in Haug et al. 1986). In *R. ochroleuca–P. abies* and *Piceirhiza nigra* the outer part of the hyphal sheath consisted of moribund hyphae and the hyphal sheath of *T. fibrillosa–P. abies* was mostly without cytoplasm. Downes et al. (1992) also found a low frequency of active hyphal sheaths in *T. fibrillosa* mycorrhizas.

After comparing results obtained by means of ICP-AES and EELS, we were able to demonstrate not only large amounts of P, K, Mg, Fe and Zn in *Xerocomus badius–P. abies* mycorrhizas, but also P and N accumulation in vacuolar bodies. The high amounts of P measured by ICP-AES may well be connected to the high number of small, well-defined osmophilic bodies in the hyphal vacuoles of the fungal sheaths. These osmiophilic bodies gave prominent P peaks in EELS. The small bodies probably represent polyphosphates which may have become aggregated during fixation. In the large, N-containing vacuolar bodies, P was frequently at the detection limit of EELS. However, measurements by means of X-ray microanalysis gave prominent P peaks (Bücking et al. 1997). The large bodies in *X. badius* mycorrhizas, therefore, also store P, as was found previously in *Cenococcum geophilum* (Kottke et al. 1995a) and mycelia of *Laccaria bicolor* (Kottke et al. 1995b). Nitrogen storage in vacuolar bodies was also reported from rhizomorphs (Franz and Acker 1995). Sclerotia of *Sclerotinia minor* Jagger accumulated both N and P in bodies which, according to the authors, were connected to the cytoplasm (Young et al. 1993). The localization of the bodies is, however, unclear in the published micrographs. Preliminary results from labelling experiments using specific antibodies indicated accumulation of amino acids in the vacuolar bodies of *X. badius* (A. Brun, personal communication). Binding to polyphosphate, as found in *Saccharomyces cerevisiae* (Dürr et al. 1979), can be assumed but needs further investigation.

In the present study, we found the binding of Al to the small P-containing bodies in field material for the first time. The ability of fungal hyphae to form Al-polyphosphate complexes has been demonstrated by in vivo NMR spectroscopy from previously in vitro-grown mycelia of *Laccaria laccata* (Martin et al. 1994). EELS enabled us to precisely localize the position of the elements in the small, osmiophilic vacuolar bodies of mycelium of *Laccaria amethystea* cultured in the same conditions as *L. laccata* (Kottke and Martin 1994). Väre (1992), working with critical-point-dried hyphae of *Suillus variegatus* (Fr.) O. Kunze and using an electron dispersion photometer connected to a scanning transmission electron microscope also found Al connected to P-containing granules. Despite criticism of the preparation methods, inevitable with transmission electron microscopical studies (Orlovich and Ashford 1993), the NMR analyses of unprepared, living hyphae and the critical-point-dried samples support the association of Al and polyphosphate in living material. There are, however, additional compartments in mycorrhizas which may bind Al, such as the cell walls or the mucigel (Horst et al. 1982). It is speculated that in *Piceirhiza gelatinosa* the mucilage may also accumulate Al, but so far no measurements have been carried out.

Results point to a link between the high vitality of the mycorrhizal sheaths formed by *X. badius*, the potential for accumulation of nutritive elements and the frequency of the N/P-containing bodies. *X. badius* mycorrhizas may then be of exceptional value to the trees on acidic stands where availability of nutrients is rather low. The fruitbodies of *X. badius* occurring frequently in coniferous and deciduous forests on acidic soils (Ceto 1987) are known to attract heavy metals like Cu or Cd (Lepsova and Mejstrik 1988) and radioactive Cs (Teherani 1987; Berreck and Haselwandter 1989). The mechanism of accumulation of heavy metals and caesium by sporocarps is still insufficiently clarified (Galli et al. 1994). We presume that accumulation of K in fruitbodies (Seeger 1978), shown here for the hyphal sheath of *X. badius* mycorrhizas, may be related to the vacuolar depositions, but K can also bind to norbadion-A, the brown pigment in the cap of *X. badius* fruitbodies (Gill and Steglich 1987). The amounts of pigment in mycorrhizas remain to be determined.

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