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Uptake and transfer of nutrients in ectomycorrhizal associations: interactions between photosynthesis and phosphate nutrition

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Abstract Energy-dispersive X-ray microanalytical investigations and microautoradiographic studies were carried out to examine whether the uptake and transfer of phosphate (P) by an ectomycorrhizal fungus is affected by the carbohydrate supply of its host plant. For this purpose, non-mycorrhizal seedlings of *Pinus sylvestris* L. and plants inoculated with the ectomycorrhizal basidiomycete *Suillus bovinus* (L. ex Fr.) Kuntze were placed in the dark for 7 days in advance of a P supply. The subcellular element distribution and the uptake and distribution of ^{33}P was analyzed in non-mycorrhizal and mycorrhizal roots of these plants and compared with plants kept constantly under normal light conditions (control plants). The results show that placing non-mycorrhizal plants in the dark in advance of the nutrient supply led to (1) a reduction of the subcellular contents of P, S and K, but to an increase in the cytoplasmic Na content, and (2) an increase of ^{33}P absorption and translocation to the shoot. It can be assumed that this increased inflow of ^{33}P in non-mycorrhizal plants was due to P starvation after suppressed photosynthesis and reduced respiration of these plants. The suppression of photosynthesis by an ectomycorrhizal host plant and the resulting lower carbohydrate supply conditions for the ectomycorrhizal fungus led to (1) a decrease of P absorption by the mycobiont, (2) a change of the P allocation in the fungal cell compartments of an ectomycorrhizal root, and (3) a reduction of P transfer to the host plant. However, microautoradiographic studies revealed that, under these conditions, P was also absorbed by the mycorrhizal fungus and translocated via the Hartig net to the host plant. In mycorrhizal roots of plants placed in the dark in advance of the nutrient supply, the cytoplasmic P content of the Hartig net was reduced and, instead, a high number of polyphosphate granules could be detected within the hyphae. The results indicate that the exchange processes between the symbionts in a mycorrhiza are possibly linked and that P uptake and translocation by an ectomycorrhizal fungus is also regulated by the carbohydrate supply of its host plant.

Keywords Carbohydrate · Ectomycorrhiza · Phosphate · *Pinus sylvestris* · *Suillus bovinus*

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Introduction

Mycorrhizal fungi are known to stimulate host plant growth mainly by enhancing soil nutrient uptake, particularly of phosphate (P). This effect has been suggested to be due to (1) an increase in the absorbing surface and the exploration of a larger soil volume by the extramatrical mycelium, (2) the small hyphal diameter leading to an increased P-absorbing surface area and, compared to non-mycorrhizal roots, higher P influx rates per surface unit, (3) the production of organic acids and phosphatases, which catalyze the release of P from organic complexes, and (4) the formation of polyphosphates (poly-P) by mycorrhizal fungi and hence low internal P concentrations (Marschner and Dell 1994). However, in a mycorrhizal infection, the efficiency of P nutrition of the host plant is not due to a higher rate of P absorption under all supply conditions but to the capacity for accumulating P under high external supply, and remobilization of this storage pool under P stress to maintain a continuous flux of P to the mycorrhizal host also under severe conditions. At sufficient and supraoptimal P supply, a mycorrhizal infection has no positive effect on P absorption (Amijee et al. 1993) and a reduction of plant growth is often observed. This can be explained by the carbon costs of a mycorrhizal infection for the host plant (Peng et al. 1993). Around 20% of the assimilated carbon from the plant is translocated to the fungal symbiont in both VA and ectomycorrhizal associations (Finlay and Söderström 1992). Jones et al. (1991), who examined P uptake concurrently with the C allocation below ground in mycorrhizal *Salix viminalis* L. cv. Bowles, reported

that mycorrhizal plants were more efficient at P acquisition per mole C expended than non-mycorrhizal plants.

It has been shown by several authors that, especially for VA mycorrhizal associations, a reduction of photon irradiance leads to a decrease in the root/shoot ratio (dry wt.), and to a reduction of the mycorrhizal growth response and the P inflow by mycorrhizal systems (Daft and El-Giahmi 1978; Tester et al. 1985). It was suggested that the decrease of the P inflow of mycorrhizal systems under photosynthesis limitation was due to higher competition between both symbionts for carbohydrates, leading to lower colonization rates of roots (Son and Smith 1988) and a decrease in the number of functional arbuscules (Hayman 1974).

The compatible interaction between mycorrhizal fungi and plants is based on a bidirectional transfer of nutrients and carbohydrates across an interface, the structure and development of which varies between different types of mycorrhizal associations. Since there is no direct symplastic continuity between both partners, nutrients must pass an interfacial apoplast before they can be absorbed (Peterson and Bonfante 1994). Models of transfer processes across this mycorrhizal interface generally involve: (1) the passive efflux of P and carbohydrates through the fungal and plant plasma membranes into the interfacial apoplast, and (2) the active absorption of nutrients by both partners driven by a H^+-ATPase (Smith et al. 1994). This protein operates as a primary transporter by pumping protons into the interfacial apoplast, thereby generating a proton-motive force, which can be used for active transport through the plasma membranes (for review, see Michelet and Boutry 1995). The normal flow of P and carbohydrates through the plasma membranes into the interfacial apoplast is calculated to be insufficient to maintain the symbiosis. Therefore, conditions in the interface that cause an enhanced efflux or a decrease in the level of competing uptake systems have been proposed by several authors (e.g. Smith et al. 1994).

At present, we have only limited information about the regulation of the transfer processes occurring across this specialized interface and the mechanisms involved in polarizing the transfer. Woolhouse (1975) and Schwab et al. (1991) assumed a coupling of the exchange processes by carbohydrate-phosphate transporters, similar to those located in the chloroplast envelope. However, based on modern molecular techniques, it has been proposed that separate membrane transport systems for the uptake of P and carbohydrates operate at the plant-fungus interface (Harrison and van Buuren 1995; Nehls et al. 1998; Rosewarne et al. 1999). In contrast to what is known for pathogens, the molecular mechanisms of nutrient transport across the mycorrhizal interface are still entirely unknown (Harrison 1999).

The aim of the current investigation was to examine P uptake by an ectomycorrhizal fungus and transfer to its host plant under different carbohydrate supply conditions to acquire further information about the exchange of nutrients between both symbiotic partners and the possible regulation of these transfer processes.

Materials and methods

Culture of plant and fungal material

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 growth chamber with a day/night rhythm of 14/10 h, a temperature of 20°C/15°C and a light intensity of 85 μmol s^{-1} m^{-2}. For experiments, the seedlings were transferred to Petri dishes (9 cm diameter) with an opening for the stem, filled with sterilized perlite. The roots were separated from the perlite by a charcoal filter paper (Schleicher & Schuell, Germany). The ectomycorrhizal basidiomycete *Suillus bovinus* (L. ex Fr.) Kuntze was originally received from R.D. Finlay, University of Lund, Sweden and cultured axenically on modified Melin Norkrans (MMN) medium (Molina and Palmer 1982) with 2% agar. The inoculation of the plants was carried out by fungal inoculum laid down in the Petri dishes close to young short roots. One week before the start of the experiment the perlite was removed from the Petri dishes to ensure establishment of new extramatrical mycelium. The plants were then separated into two treatments. The plants of the first fraction were subsequently cultured under normal light conditions (controls), the second fraction was placed in the dark for a period of 7 days in advance of the labelling experiment (dark treatment).

Labelling of the plants with ^{33}P

The P uptake experiments were carried out by using ^{33}P-inorganic phosphate (P_i) as a radioactive tracer. Based on the low maximum energy of the emitted negatrones (0.25 MeV), which is comparable to ^{14}C, this isotope is also suitable for microautoradiographic investigations and allows a good spatial resolution of the autoradiographs. For this purpose, 7.4×10^5 Bq $^{33}P_i$ (20 μCi, 0.128 ng ^{33}P) was added to a nutrient solution based on that of Ingestad (1960) without addition of P (1.785 mM NH_4NO_3, 0.624 mM KCl, 1 mM $CaCl_2$, 0.63 mM $MgSO_4$, 0.017 mM Fe-EDTA, 3 μM $MnCl_2$, 0.015 mM H_3BO_3, 3 μM $ZnCl_2$, 3 μM $CuCl_2$, 0.03 μM Na_2MoO_4). The growth chambers were watered with this nutrient solution and the labelling of the plants was carried out under normal light conditions. Needles of all plants and cuttings of the mycorrhizal root systems (non-mycorrhizal root branches with mycorrhizal root tips) were harvested after 4 and 6 days for liquid scintillation counting and microautoradiographic studies. Since non-mycorrhizal root systems had only small numbers of short roots, samples of these roots were taken only after 6 days. The samples were washed in a non-labelled nutrient solution and dried in an oven for liquid scintillation counting. For microautoradiographic studies the washed roots were immediately cryofixed by plunging into subcooled nitrogen.

Supply of stable phosphate

To obtain information on the effect of the dark treatment on the subcellular element distribution, some plants of both treatments were supplied with stable P as KH_2PO_4 at the same concentration used in the radiolabelling experiment (0.128 ng P). After 6 days, non-mycorrhizal and mycorrhizal roots were harvested and prepared for energy-dispersive X-ray spectroscopy (EDXS). Additionally, cuttings of the mycorrhizal root systems (non-mycorrhizal root branches with mycorrhizal root tips) were harvested to determine the soluble carbohydrate content of these roots.

Liquid scintillation counting

The samples were dried, weighed and digested with a tissue solubilizer (Soluene 350, Packard). After digestion, the samples were suspended in a scintillation cocktail (Optiphase, Wallac, Finland). Radioactivity was determined by liquid scintillation counting (Microbeta Trilux, Wallac, Finland) with correction of the counting accuracy using an internal standard.

Sample preparation for EDXS and microautoradiography

The samples were cryofixed by plunging into subcooled nitrogen and freeze-dried under low temperature and high vacuum conditions (CFD, Leica, Germany). To prevent recrystallization of water in the plant tissues, the freeze-drying was started at –100°C for 7 days and continued for 2 days each at –90°C, –80°C and –60°C, respectively. The samples were then slowly warmed up to room temperature, followed by either pressure-infiltration according to a method described by Fritz (1980) directly in 100% epoxy resin (Spurr 1969) for EDXS or by treatment with dried diethyl ether as an intermedium in successive preliminary steps (100%; 1:4, 2:1, 4:1 v:v) for microautography. After polymerization, dry sections were cut with a glass knife.

Microautoradiography

The microautoradiographs were carried out by coating dry 1 µm sections with a thin layer of the nuclear research emulsion L4 (Ilford, Germany) with a crystal size of 0.11 µm. The thickness of the dried layer was approximately 5 µm. After exposure at 4°C for 4 months, the film was processed with the fine grain developer D19 A/S (Sanderson 1981), rinsed in demineralized water and then fixed with a commercial black-and-white fixer (Tetenal, Germany). As a control, slides without sections and with unlabelled root sections were also processed in the same way as described for the labelled samples. On these slides almost no silver grains were visible with Nomarski DIC microscopy.

EDXS microanalysis

Dry sections of 500 nm were transferred to filmed folding Cu-grids and carbon coated (20 nm). The X-ray microanalytical studies were carried out under standardized conditions using the electron microscope Philips EM 420 equipped 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 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}) was 12 nm, but it can be assumed that the real spot size was slightly larger due to interactions of the primary electron beam with the specimen. The element distribution is documented as a peak to background (P/B) ratio in order to diminish the effects of surface irregularities of the sections during analysis. Due to the difficult standardization of the X-ray microanalytical technique, the P/B ratio is used as a semi-quantitative measurement of the element level in different cellular compartments. Of the harvested roots, five were dry cut and, within each of these, three spectra of every cellular compartment of different randomly chosen cells were analysed (n=15). The apoplastic element levels represent measurements in transitional zones between different root tissues to prevent distortion of the results by neighboring cytoplasmic regions.

Carbohydrate analysis

Cuttings of the mycorrhizal root systems were dried in an oven at a temperature of 70°C, weighed and digested with hydroxylammonium chloride (25 mg ml^{-1} in pyridine) for 1 h at 80°C. The samples were then silyated using hexamethyldisylazane and trifluoroacetic acid for 30 min at 80°C. The soluble carbohydrate content was determined by a gas chromatograph (HP 6890) equipped with a cold injection system and a mass selective detector (HP 5972) under the following conditions: column: 29 m ×250 µm, df =0.25 µm; temperature: 50–300°C; carrier gas: He, 0.7 bar. Retention times under these conditions were 17.15 min, 17.53 min, and 18.33 min for the C6 carbohydrates mannitol, fructose and glucose, respectively, and 28.25 min for the C12 carbohydrate sucrose. The fungal carbohydrate trehalose could not be detected under these conditions, i.e., the trehalose content in most of the samples was under the limit of detection.

Statistical treatment

In the figures, mean value and confidence intervals are given as vertical bars ($P \leq 0.05$). The results are only discussed if no overlap of the intervals was observed, or if a statistically significant difference between the treatments by the non-parametric U-test of Wilcoxon, Man & Whitney (Unistat) was found. This is indicated in the figures as letters on the bars.

Results

Subcellular element distribution in non-mycorrhizal roots

Placing plants in the dark in advance of a P and nutrient supply had a clear effect on the subcellular content of different elements measured by EDXS (Fig. 1). The P, S and K content in different cellular compartments were clearly reduced, whereas the Na content was increased by the dark treatment. After dark treatment in non-mycorrhizal roots, P and S could be detected mainly in the cytoplasm and apoplast of the stele, whereas the content of these elements in outer root compartments was significantly lower (Fig. 1a, b). In contrast, control plants showed high P and S content also in the root cortex, here especially in the cytoplasm and apoplast. The vacuolar contents were generally lower and the differences between the various treatments were smaller. The K distribution showed a typical radial gradient with the highest K content in the cytoplasm and apoplast of the stele (Fig. 1c). The dark treatment of the plants led to a reduction of the K content especially in apoplastic regions and vacuoles of the cortex, whereas the cytoplasmic K level in different regions of the root was not affected. In contrast, the Na content, especially in cytoplasmic and apoplastic root compartments, was increased by the dark treatment whereas the vacuolar content tended to be higher in controls (Fig. 1d).

The effect of an ectomycorrhiza on the subcellular P distribution in roots

The dark treatment had an influence on the P content in the fungal and plant cell compartments of mycorrhizal roots. The P content in the cytoplasm of the fungal sheath and the Hartig net was clearly reduced by placing mycorrhizal plants in the dark (Fig. 2). A slight decrease of the P content after dark treatment was also detectable in the interfacial apoplast (not significant on the 5% level, P =0.09). The P content in the cell compartments of the stele was also reduced, whereas that in the root cortex was not affected by the dark treatment.

The reduction of the P content in the fungal cell compartments of mycorrhizal roots after dark treatment was not due to a decrease of the total intracellular P concen-

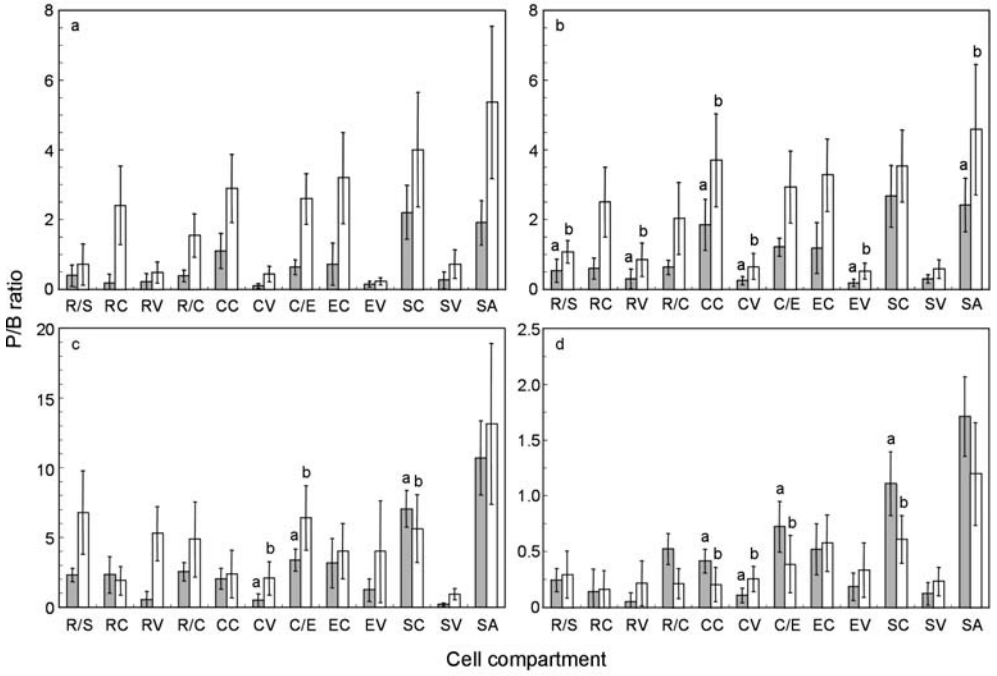

Fig. 1 Subcellular distribution of P compared to S, K and Na (**a**), S (**b**), K (**c**) and Na (**d**) in non-mycorrhizal roots depending on the light treatment in advance of the nutrient supply (*white bars* control plants, *grey bars* 7 day dark treatment). *R/S* Outer tangential cell wall of the epidermis, *RC* epidermis cytoplasm, *RV* epidermis vacuole, *R/C* apoplast between epidermis and cortex, *CC* cortex cytoplasm, *CV* cortex vacuole, *C/E* apoplast between cortex and endodermis, *EC* endodermis cytoplasm, *EV* endodermis vacuole, *SC* stele cytoplasm, *SV* stele vacuole, *SA* stele apoplast. Mean values (n =15) of the peak to background (P/B) ratios ± confidence intervals as vertical bars ($P \leq 0.05$) are given. The different letters on the bars show significant differences between mean values of one cellular compartment calculated by the non-parametric U-test of Wilcoxon, Man and Whitney ($P \leq 0.05$)

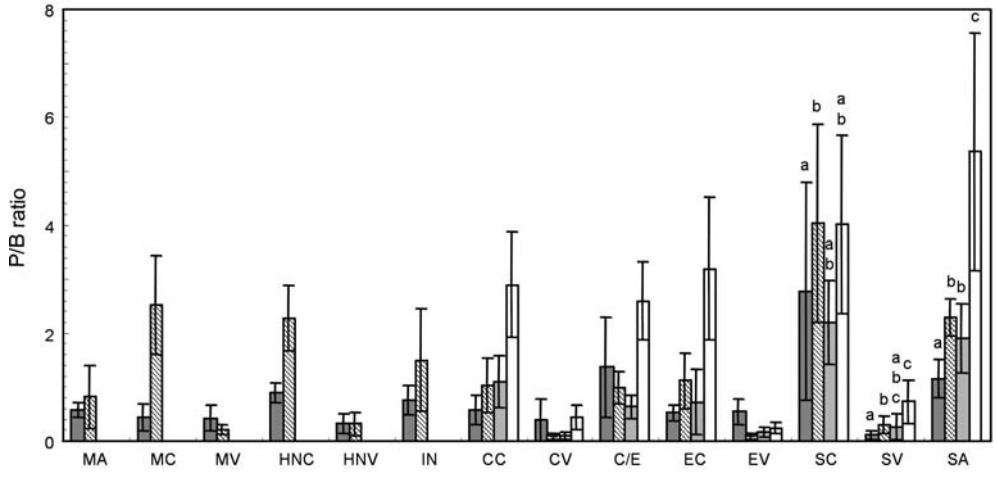

Fig. 2 Subcellular distribution of P in non-mycorrhizal (*NM*) and mycorrhizal (*M*) roots depending on the pretreatment in advance of the P supply (control plants: *hatched bars* M, *white bars* NM; 7 day dark treatment: *dark grey bars* M, *light grey bars* NM). *MA* Mantle apoplast, *MC* mantle cytoplasm, *MV* mantle vacuole, *HNC* Hartig net cytoplasm, *HNV* Hartig net vacuole, *IN* interfacial apoplast, *CC* cortex cytoplasm, *CV* cortex vacuole, *C/E* apoplast between cortex and endodermis, *EC* endodermis cytoplasm, *EV* endodermis vacuole, *SC* stele cytoplasm, *SV* stele vacuole, *SA* stele apoplast. Mean values (n =15) of P/B ± confidence intervals as vertical bars ($P \leq 0.05$) are given. The different letters on the bars show significant differences between mean values of one cellular compartment calculated by the non-parametric U-test of Wilcoxon, Man and Whitney ($P \leq 0.05$)

tration of the fungal hyphae. Rather, the allocation between different P pools within the fungal hyphae appeared to be affected. In the fungal vacuoles of the sheath and the Hartig net, numerous electron opaque granules were found after dark treatment (Fig. 3a), which contained high amounts of P (Fig. 3b). It can be assumed that these granules were poly-P granules associated with the cations K^+ (Fig. 3c), Mg^{2+} (Fig. 3d) and Ca^{2+} (not shown). Poly-P granules were also found in ectomycorrhizal roots of control plants, where the number appeared to be lower. An exact quantification was, however, not

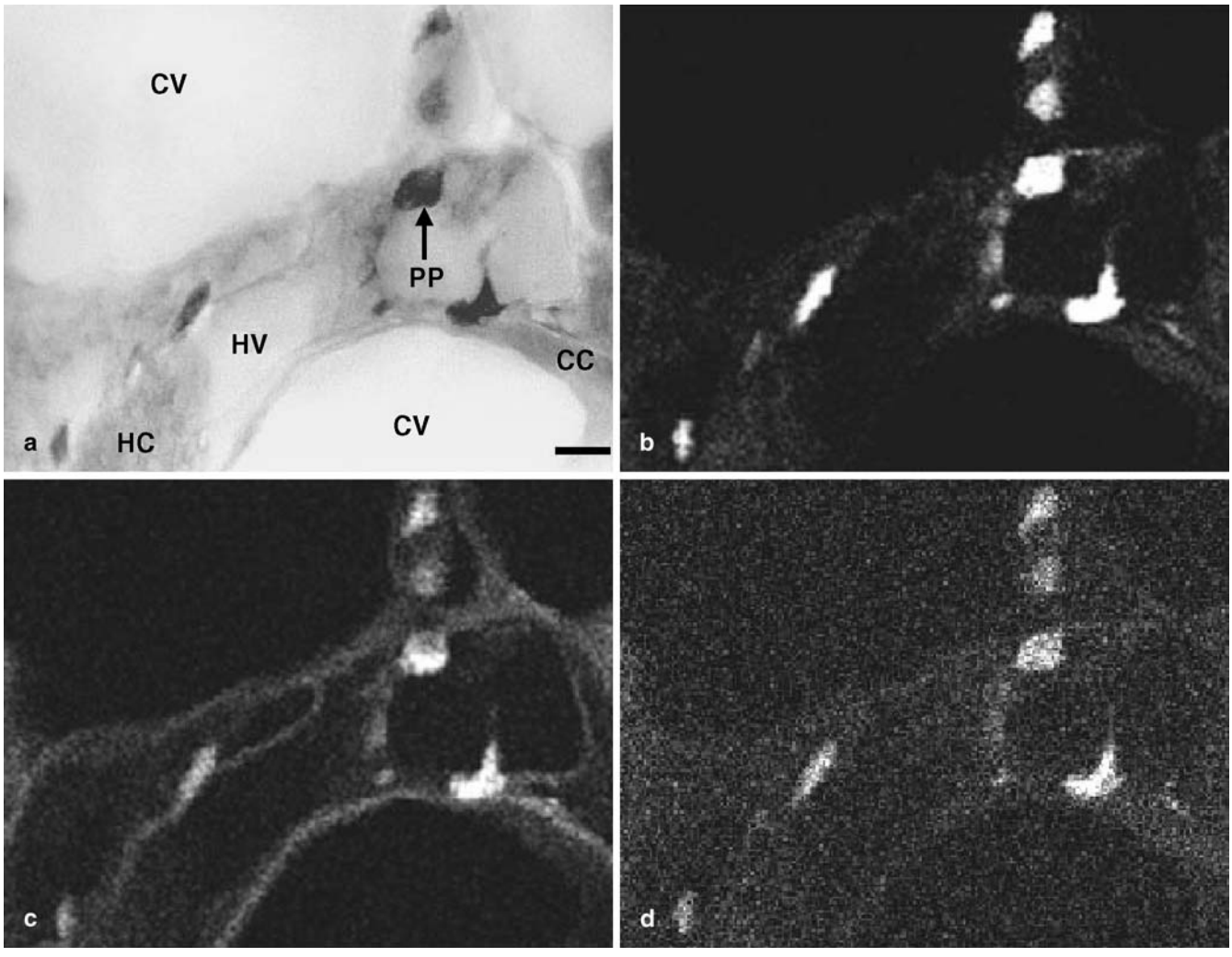

Fig. 3 **a** Polyphosphates (poly-P) within the Hartig net of a *Suillus bovinus* ectomycorrhizal pine seedling that was placed in the dark for 7 days in advance of the P_i supply (*scale bar* 1 μm). Elemental map of specific P (**b**), K (**c**) and Mg (**d**) accumulations in electron-opaque poly-P granules. *CC* Cortex cytoplasm, *CV* cortex vacuole, *HC* fungal cytoplasm, *HV* fungal vacuole, *PP* poly-P

possible, since semithin sections at high magnification (6,400 or 12,500×) were used and the display window of the STEM unit was very small.

Mycorrhizal formation had an effect on the P content of different cellular compartments. In mycorrhizal roots of control plants, a lower P content was found in cortex and stele than in non-mycorrhizal roots (Fig. 2). However, mycorrhizal and non-mycorrhizal roots did not differ in their P content after dark treatment.

Uptake and distribution of ^{33}P in non-mycorrhizal and mycorrhizal plants

The uptake and distribution of ^{33}P in non-mycorrhizal and mycorrhizal plants was affected by dark treatment in advance of the radiolabelling (Table 1). Placing plants in the dark in advance of the ^{33}P supply led to an increase of P absorption by non-mycorrhizal plants, but reduced

Table 1 Distribution of phosphate (P) (in dpm) in non-mycorrhizal and *Suillus bovinus*-inoculated seedlings of *Pinus sylvestris*. *Light* Normal light conditions, *dark* 7 day dark treatment in advance of the $^{33}P_i$ application. Mean value (n=10) ±SE

	Non-mycorrhizal plants		*S. bovinus*-inoculated plants	
	Light	Dark	Light	Dark
Non-mycorrhizal roots (6 days)	47,643±9,627	116,121±22,911	–	–
Mycorrhizal roots (4 days)	–	–	39,696±7,616	34,291±2,887
Mycorrhizal root (6 days)	–	–	81,037±25,888	60,376±10,377
Needles (4 days)	442±162	1,934±509	1,630±479	797±175
Needles (6 days)	1,296±452	5,188±1749	2,259±709	1,330±621

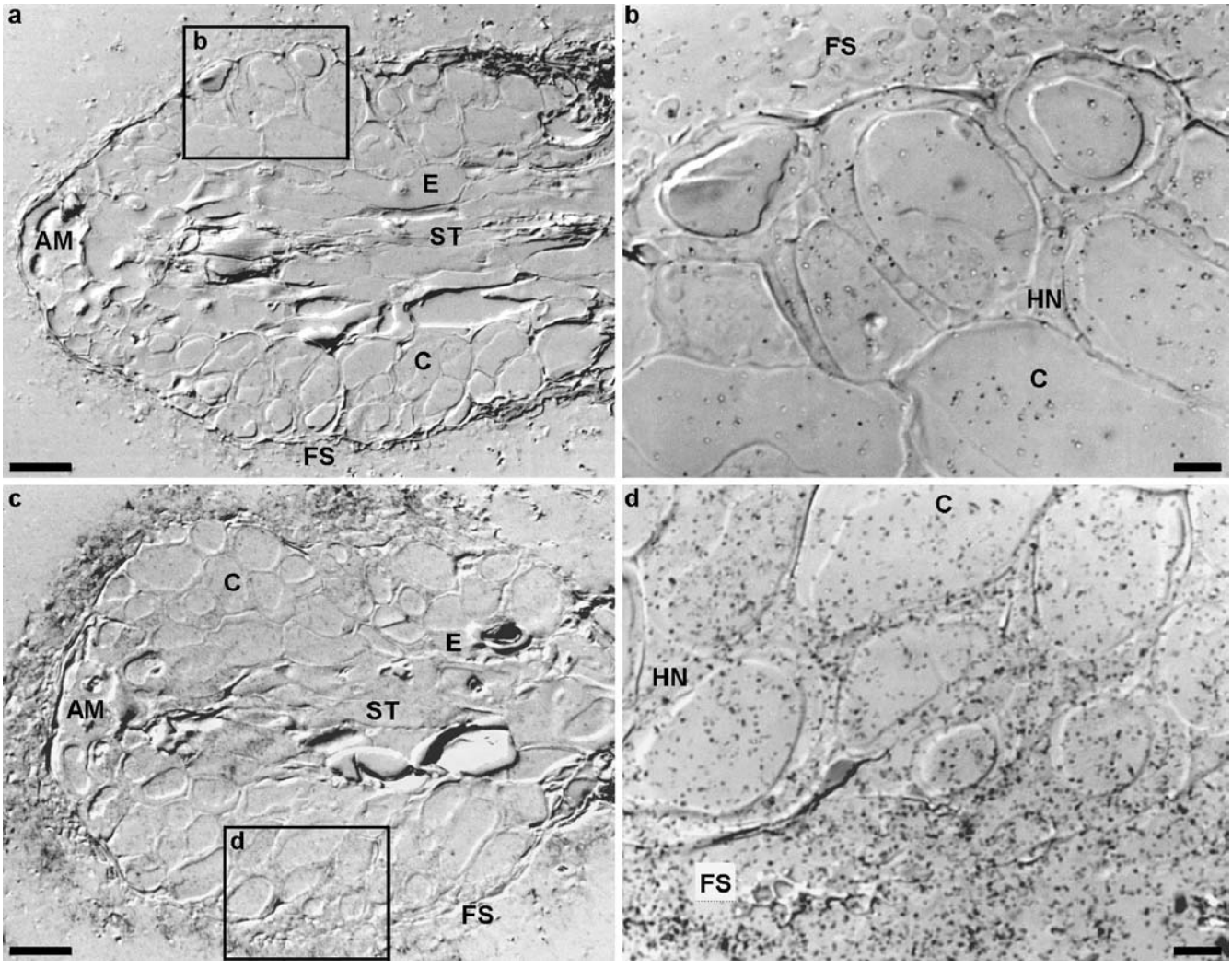

Fig. 4 Microautoradiographs (longitudinal sections) of mycorrhizal roots harvested 6 days after $^{33}P_i$ application. **a**, **b** Control plants kept constantly under normal light conditions; **c**, **d** 7 day dark treatment in advance of the $^{33}P_i$ application. *AM* Apical meristem, *C* cortex, *E* endodermis, *FS* fungal sheath, *HN* Hartig net, *ST* stele. *Scale bar* 100 μm (**a**, **c**), 20 μm (**b**, **d**)

the uptake by mycorrhizal seedlings. This effect could be observed in both in the root and in the needle fraction. Mycorrhizal control plants showed a higher level of ^{33}P absorption by their roots and greater translocation of ^{33}P via the stem to their needles than non-mycorrhizal plants, whereas after dark treatment higher contents of ^{33}P in root and shoot of non-mycorrhizal plants were detected.

After dark treatment, only a small number of silver grains could be observed on the sections, indicating low ^{33}P absorption by mycorrhizal roots during radiolabelling (Fig. 4a, b). The ^{33}P was fairly homogenously distributed among both symbiotic partners, and the silver grains, which were visible in the Hartig net and the cortical region, showed that also after dark treatment ^{33}P was absorbed by the mycorrhizal fungus and translocated via the hyphae to the host plant. In contrast, mycorrhizal control plants showed a higher level of ^{33}P absorption and therefore a higher silver grain density than plants after dark treatment (Fig. 4c, d). The labelling in these roots was not homogenously distributed between both symbiotic partners. The labelling was denser compared to the neighbouring root cells, especially in the fungal sheath (Fig. 4c) and in the Hartig net (Fig. 4d). The dense labelling in the cortical cells showed that under normal light conditions more ^{33}P was absorbed by the mycorrhizal fungus and transferred to the host plant.

Soluble carbohydrate content in mycorrhizal roots

The contents of the carbohydrates sucrose, glucose, fructose and mannitol in mycorrhizal roots are shown in Table 2. In plants, sucrose is the main form in which carbohydrates are translocated, mannitol is fungal, and glucose and fructose are present in both symbionts. The results indicate that a dark treatment of the host plant in advance of the nutrient supply led to a reduction of the soluble carbohydrate content in mycorrhizal roots. In particular, the content of the hexose glucose and of the fungal carbohydrate mannitol was clearly reduced by the dark treatment.

Table 2 Soluble carbohydrate contents (in µg/mg dry wt.) in mycorrhizal pine roots depending on the pretreatment. *Light* Normal light conditions, *dark* 7 day dark treatment in advance of a nutrient supply. Mean value (n =3) ±SE

Carbohydrate	Light	Dark
Sucrose	0.752±0.243	0.554±0.163
Glucose	0.437±0.068	0.151±0.053
Fructose	0.791±0.144	0.515±0.163
Mannitol	0.534±0.420	0.035±0.004

Discussion

The effect of dark treatment on subcellular element distribution in non-mycorrhizal roots

The element distribution in non-mycorrhizal roots measured by EDXS was clearly affected by dark treatment. It can be supposed that the reduction in the P, S and K content after dark treatment was due to lower respiration rates and a reduced content of the ATP needed for active transport processes through the plasma membranes. The absorption of these nutrients is an active process driven by a plasma-membrane-located H^+-ATPase that pumps protons into the apoplast thereby generating a proton electrochemical potential gradient across the plasma membrane, which can be used for active transport of these nutrients (Lüttge and Clarkson 1989; Mimura 1999; Smith 2001). On the other hand, the uptake of Na, which is not an essential element, except in certain salt-tolerant plants with C_4 and CAM photosynthesis (Clarkson and Hanson 1980), is normally limited, and the cytoplasmic concentration is actively regulated by exclusion or compartmentation in the vacuoles by Na^+/H^+-antiports (Spickett et al. 1993). This can explain why, after dark treatment, the cytoplasmic and vacuolar Na content was higher and lower, respectively, than in the control plants.

It has often been reported that a reduction in photon irradiance reduces P and K inflow of non-mycorrhizal plants (e.g. Hunt and Burnett 1973; Tester et al. 1985). At low photon irradiance, the proportion of carbohydrates transferred to the root is normally reduced. This can be interpreted as a strategy to increase the proportion of the biomass involved in photosynthesis when plants are light-limited (Smith and Gianinazzi-Pearson 1990).

Temporary placement of plants in the dark led to a decrease of the P and S content in all cell compartments, especially in the cytoplasm of the root cortex. After dark treatment, a high content of these elements was found only in the apoplast and the cytoplasm of the stele. It can be assumed that the dark treatment led to a reduction of active uptake processes, thereby causing severe P- and S-starvation of the plants. In plants, P_i is distributed between cytoplasmic, metabolically active, and a vacuolar, metabolically inactive, storage pools. Lee and Ratcliffe (1993) reported that the cytoplasmic P_i content was relatively constant over a wide range of external P concentrations, although severe P_i deficiency also caused a decrease in the metabolically active pool of maize roots. In contrast, the vacuolar P_i pool depends on the external P supply (Lee and Ratcliffe 1993; Bücking and Heyser 2000) and is proposed to act as a buffer to supply to the cytoplasm or to sequester P_i in varying P conditions.

The effect of dark treatment on ^{33}P uptake by non-mycorrhizal roots

The dark treatment led to a clear reduction of the P content in non-mycorrhizal roots but increased the uptake of P after supply of ^{33}P under normal light conditions. This confirmed our previous results (Bücking and Heyser 2001) that non-mycorrhizal seedlings of *Populus tremula* × *Populus alba* L., placed in the dark in advance of a ^{33}P-labelling, showed an increased accumulation of P in their root systems. It was suggested that this effect might be due to a reduction of the P demand of the shoot. P is an essential element for photosynthesis and starch metabolism, and uptake and distribution of P in plants is normally regulated by the demand for P – especially in the plant parts above ground (Drew and Saker 1984). However, in the experiments presented here, higher ^{33}P-contents were found also in the needle fraction after dark treatment. Based on the X-ray microanalytical results it can be assumed that the plants were P-deficient after a period of 7 days in the dark. The higher uptake under conditions of P deficiency might be due to induction of genes encoding P transporters. These are transcriptionally regulated by feedback signals that respond to the P status of the plant (Muchal et al. 1996; Liu et al. 1998b). The genes encoding high affinity P transporters are expressed mainly in cells in close contact with soil solution, especially epidermal cells with their associated root hairs, whereas lower affinity transporters are expressed in cells associated with the vascular system, where they might be involved in the internal redistribution of P (Smith 2001).

The results showed that after a dark period of 7 days, 6 days under normal light conditions and increased P absorption was not long enough to equalize the differences in the subcellular P contents between non-mycorrhizal short roots of plants after dark treatment and control plants. This is possibly due to the function of non-mycorrhizal short roots within the root system of pines and to the fast allocation of the absorbed P within the plant after P starvation. It was assumed that non-mycorrhizal short roots do not act primarily as nutrient-absorbing organs but rather as a carbohydrate source for potential fungal symbiotic partners (Read 1992). Under P starvation, nutrient-absorbing parts of the root system may act as possible sinks for P. Under P deficiency, the P content of the shoot is normally reduced to a greater extent, and faster, than the root content. It has been concluded that growth of the root and the resulting extension of the absorbing surface at the expense of further shoot growth is a strategy of plants under P limitation (Clarkson 1985).

Split root experiments indicate that gene expression of P transporters responds to the overall P status of the whole plant and is not regulated by the localized concentration of P adjacent to that particular root (Liu et al. 1998a).

The effect of dark treatment on P uptake and translocation by an ectomycorrhizal fungus

In ectomycorrhizal pine seedlings that showed higher contents of ^{33}P in roots and needles than non-mycorrhizal plants under normal light conditions, ^{33}P absorption was clearly reduced by dark treatment of the host plant. A reduction of the P inflow in VA mycorrhizal associations by a decrease of the photon irradiance has often been described (e.g. Tester et al. 1985; Son and Smith 1988; Smith and Gianinazzi-Pearson 1990). Bücking and Heyser (2001) found that placing mycorrhizal poplar seedlings in the dark in advance of a ^{33}P supply reduced the P content in mycorrhizal roots and the P transfer to the shoot. Son and Smith (1988) suggested that this effect might be due to a reduction of the colonization rate of the root system, so that hyphal connections with the soil and the area of contact between the symbionts would have been less, leading to a lower contribution of the hyphal pathway to uptake. A reduction of fungal activity as a result of competition between the symbionts for energy supply, with the allocation of photosynthate to fungal growth rather than to the nutrient transport processes, could also be an explanation for this effect (Son and Smith 1988).

The present investigation showed that the availability of photosynthates for the fungal symbiont decreased as a result of dark treatment of its host plant. The microautoradiographic results showed that, at the same time, P uptake by the fungal hyphae, translocation, and transfer to the host plant is reduced when the carbohydrate supply for the mycorrhizal fungus decreases. X-ray microanalysis revealed a reduction in the P content in the cytoplasm of the Hartig net and the fungal sheath. It has been suggested that P transfer to the host plant is regulated by the intracellular P concentration in the hyphae of the Hartig net (Cairney and Smith 1993; Bücking and Heyser 2000); high P concentrations might increase the efflux of P from the hyphae into the interfacial apoplast, reduce reabsorption by the mycorrhizal fungus and increase the rate of translocation to the mycorrhizal host plant. Furthermore, a transfer of P across the ectomycorrhizal interface would (1) reduce the intracellular P concentration in hyphae of the Hartig net, (2) stimulate the flux of P from the extramatrical hyphae to the Hartig net, and (3) thereby increase P absorption by the extramatrical mycelium. It has been shown that in regions of the root where the highest transfer of P to the host plant takes place, the highest P inflow by the fungal sheath is also localized (Bücking and Heyser 1997). Maldonado-Mendoza et al. (2001) showed that a P transporter gene (GiPT) from the extra-radical mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* (Schenk and Smith) is regulated in response to P in the environment. The data also indicated that the P status of the mycorrhizal root and/or of intra-radical fungal structures influences P uptake by the extra-radical hyphae and the expression of GiPT. It has been concluded that the P demand of the host plant regulates P uptake by a mycorrhizal fungus (Cairney and Smith 1992). Regulation by the host would ensure that the mycorrhizal root absorbs P with the greatest efficiency when the plant is under P limitation (Thomson et al. 1990). The expression of P transporter genes of the plant is suppressed in response to colonization by a VA-mycorrhizal fungus (Burleigh and Harrison 1997; Chiou et al. 2001). However, the results showed that, even if the plant was P deficient, P absorption and transfer by the mycorrhizal fungus to its host plant was reduced when photosynthetic activity and transfer of carbohydrates to the fungal symbiotic partner was low. Under these conditions, the cytoplasmic P content of the Hartig net was reduced and instead a high number of poly-P granules could be detected within the hyphae. The functions of poly-P are: (1) P storage in vacuoles, (2) regulation of the levels of ATP and other nucleoside triphosphates, (3) homeostasis of cations, and (4) participation in membrane transport processes, cell wall formation and gene expression (Kulaev et al. 1999). The presence of poly-P granules in the fungal sheath and the Hartig net indicated that the fungus was not P- or ATP-deficient, and this might be not an explanation for the decrease in P absorption and transfer to the host plant. It has been proposed that poly-P granules are an artifact of specimen preparation and that in living hyphae poly-P of only short chain length exists (Orlovich and Ashford 1993). In contrast, Bücking and Heyser (1999) reported that these granules are actually present in living hyphae and are not caused by fixation or staining of cells. The results showed that a high proportion of the intracellular P accumulated in the fungal vacuole and the cytoplasmic P content of the Hartig net was concomitantly reduced. A possible explanation for this finding could be an interaction of the exchange processes in the mycorrhizal symbiosis. It was suggested by Harley and Smith (1983) that the carbohydrate uptake into the fungus might be coupled to the breakdown of poly-P in the fungal hyphae, thereby leading to higher intracellular P concentrations and higher efflux rates into the interfacial apoplast. It was shown for the endophyte *Gigaspora margarita* Becker & Hall by Solaiman and Saito (2001) that both glucose and its analogue, 2-deoxyglucose enhanced P efflux and that the poly-P content decreased at the same time. Also, the disaccharide sucrose, which was assumed to be the carbohydrate translocated through the plant plasma membrane into the interfacial apoplast (Smith and Read 1997), has been shown to induce a higher P efflux through the plasma membranes of ectomycorrhizal fungi (H. Bücking, unpublished data).

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