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ORIGINAL PAPER

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Phosphate uptake and polyphosphate metabolism of mycorrhizal and nonmycorrhizal roots of pine and of *Suillus bovinus* at varying external pH measured by in vivo ^{31}P-NMR

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Abstract Comparative in vivo ^{31}P-NMR analyses of mycorrhizal and nonmycorrhizal roots of *Pinus sylvestris* and the fungus of *Suillus bovinus* in pure culture were used to investigate alterations in phosphate metabolism due to changes in external pH in the range 3.5–8.5. All control samples maintained a constant pH in both cytoplasm and vacuole. Mycorrhizal roots and pure fungus, but not nonmycorrhizal roots, transformed accumulated inorganic phosphate into mobile polyphosphate with a medium chain length. Phosphate uptake rates and polyphosphate accumulation responded differently to external pH. In all cases, maximal phosphate uptake occurred at an external pH close to 5.5. At an external pH of 8.5, both roots and fungus showed a distinct lag in phosphate uptake, which was abolished when the external pH was lowered to 7.5. An irreversible effect on phosphate uptake as a consequence of variation in external pH was also observed. The central role of the fungus in regulating mycorrhizal phosphate metabolism is discussed.

Key words External pH · NMR · Phosphate · Polyphosphate

Introduction

The pH of the soil solution, particularly its acidity, may be an important factor in the decline in vitality of many forest trees through effects on mycorrhizas. This contributes to forest decline as a multiple stress disease (e.g. Manion 1981). Ectomycorrhizal trees often show better growth and improved resistance against unfavourable conditions than nonmycorrhizal trees (Harley and Smith 1983). Many reports suggest that soil pH is closely connected to effects of toxic ions, e.g. metal toxicity, upon fungi and nonmycorrhizal roots (Colpaert and VanAssche 1992; Turnau and Kozlowska 1991; Turnau et al. 1993), but detailed information about primary effects of pH on mycorrhiza function is lacking.

Although many studies have been published on the uptake and storage of phosphate by mycorrhizas (e.g. Finlay and Read 1986; Grellier et al. 1989; MacFall et al. 1992), nonmycorrhizal roots (e.g. Lee and Ratcliffe 1983; Pfeffer et al. 1987), and mycorrhizal fungi (e.g. Cairney et al. 1988; Martin et al. 1985; Mousain and Salsac 1985), information about the effect of pH on this aspect of metabolism is generally lacking.

Investigations of the influence of varying external pH on internal cytoplasmic and vacuolar pH have been carried out with mung bean root tips using ^{31}P-NMR (Torimitsu et al. 1984). The results showed only small internal pH shifts resulting from a change of external pH from 3 to 10. It has previously been shown (Gerlitz and Werk 1994) that the ectomycorrhizal fungus *Suillus bovinus* has a vacuolar pH of 5.4 ± 0.1 (0.7 ppm P) and a cytoplasmic pH of 6.5 ± 0.1 (1.3 ppm P). For the roots of *Pinus sylvestris* the same study gave values of pH 6.1 ± 0.1 (1.0 ppm P) for the vacuole and pH 7.7 ± 0.1 (2.6 ppm P) for the cytoplasm (Gerlitz and Werk 1994).

The first aim of the current study was to investigate the effect of change in pH in the nutrient medium on the cytoplasmic and vacuolar pH of *S. bovinus* and of mycorrhizal and nonmycorrhizal excised roots of *P. sylvestris.* The second aim was to investigate whether phosphate uptake and polyphosphate metabolism of the samples investigated is affected by change in external pH in the range 3.5–8.5.

Materials and methods

Cultures

Nonmycorrhizal and mycorrhizal seedlings of *P. sylvestris* were grown initially in a sterile environment using autoclaved sand,

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Hoagland's solution (Hoagland and Arnon 1950) as a nutrient, and a light/dark cycle of 14/10 h. After about 2 months, the seedlings were split into two groups, one remaining in the sand and the other prepared for in vitro mycorrhizal inoculation.

In vitro mycorrhizal inoculation was performed using Petri dishes with carbon filters fixed to the inside of one half (Kottke et al. 1987). Seedlings of *P. sylvestris* were passed through a slit in the rim of each Petri dish. Before closing the dishes, the roots were inoculated with *S. bovinus* (Kottke et al. 1987) and the plates then filled with sand. The seedlings were grown vertically in the conditions described above for about 2 months.

Cultures of *S. bovinus* were obtained from Dr. R. D. Finlay, Department of Microbiological Ecology, University of Lund, Sweden. The mycelia were grown for 3.5 weeks in NMN liquid medium (Modified Melin-Norkrans solution) (Marx and Bryan 1975) at pH 5.5, producing a dry weight of 150–250 mg and a diameter of 4–5 cm.

NMR techniques

^{31}P-NMR investigations were performed using a Bruker AM 360-FT spectrometer and a 20-mm diameter tube. The conditions established for NMR experiments were defined in a previous paper (Gerlitz and Werk 1994). The internal pH was determined from a an in vitro pH versus chemical shift plot using a phosphate solution (Lee and Ratcliffe 1983; Roberts et al. 1980, 1981).

Phosphate uptake and polyphosphate metabolism

Investigations on the external pH dependence of phosphate uptake and storage were performed on *S. bovinus,* and on mycorrhizal and nonmycorrhizal excised roots of *P. sylvestris.* All the experiments were performed in triplicate. Defined nutrient solution conditions were maintained by inserting two flexible tubes into the NMR tube. The solution to be monitored was transported in and out of the NMR tube by a pump at a rate of 500 ml/h.

Before starting, all samples were pretreated as follows. To remove adhering sand and avoid large line widths, the excised roots were rinsed with demineralized water and then transferred into the NMR tube. All the NMR investigations began at the same time with a wash phase. To ensure uniform conditions, the pure mycelium was handled in the same way. The wash phase was completed within 6400 scans. The nutrient supply was started by alternating between two forms of Hoagland's solution adjusted to pH 5.5, one containing 0.1 mM KH_2PO_4 and the other without phosphate. Periodic switching between the solutions at intervals of 5 and 33 min resulted in a cyclic supply. After about eight supply cycles, metabolic steady state conditions were reached, i.e. conditions of cyclic reproducible in vivo ^{31}P-NMR spectra (for more details see Gerlitz and Werk 1994).

External pH range was varied over two subranges, a lower range of 6.5, 5.5, 4.5, 3.5, 4.5, 5.5, 6.5, and an upper range of 6.5, 7.5, 8.5, 7.5, 6.5. External pH was changed by preparing supply pools filled with unbuffered Hoagland's solutions at the respective pH adjusted by 1 M HCl and NaCl.

After steady state conditions were reached, the investigation was started using the experimental external pH sequence and running a number of 5 supply cycles at each external pH. During the cyclic nutrient supply, $5\times10\times7=350$ and $5\times10\times5=250$ ^{31}P spectra of 3.8 min each were recorded in the NMR spectrometer for each pH range. Thus, 10 spectra over a period of 38 min formed one block, corresponding to one complete nutrient supply cycle during the same time. A total of 35 blocks carried out in this way constituted one experiment using the lower external pH range of seven different pHs, and 25 blocks in the upper external pH range of five different pHs.

After change in external pH, a period of about 30 min was required to reach a new cyclic reproducible metabolic state. Thus, for each pH change the first 10 spectra (i.e. the first block) was not taken recorded. The spectra corresponding to the same time period, (i.e. 4 identical types of spectra) were computerized. This procedure increased the signal to noise ratio by 2 and resulted in 10 different spectra corresponding to $4\times400\times1600$ scans each. Calculation of the means of each of the 10 summed spectra gave "block averaging". Equal treatment of all the spectra taken by NMR finally led to 7 sets and 5 sets of spectra for the upper and lower pH ranges, respectively, each set reflecting the maximal levels of uptake and storage of phosphate by the sample at each of the different external pHs.

Integration of the peaks from the ^{31}P-NMR spectra reflected internal concentrations of PP (mobile polyphosphate of medium- to long chain length) and P_i (inorganic phosphate) (for details, see Gerlitz and Werk 1994). The areas of PP_4 (middle phosphate groups of PP) and P_i were taken to estimate relative intracellular concentrations in arbitrary values, each data point corresponding to an internal concentration of P_i and PP up to a maximum for each nutrient supply cycle (see Fig. 1). Information about the mobile PP was obtained by integrating the PP_4 peak, which is characteristic of medium-chained phosphates of polyphosphate. In investigations of the influence of external pH on phosphate metabolism, on the maximal levels of P_i and PP in each cycle (see Fig. 1) were taken into account.

The validity of the NMR method for such kinetic studies was verified by comparing phosphate concentrations of inflow and effluent of the NMR tube.

Results

Internal oH

The in vivo spectra of pure mycelium of *S. bovinus,* and of mycorrhizal and nonmycorrhizal excised roots of *P. sylvestris* indicated that the chemical shifts of both vacuolar and cytoplasmic inorganic phosphate were constant with change in external pH in the range 3.5–

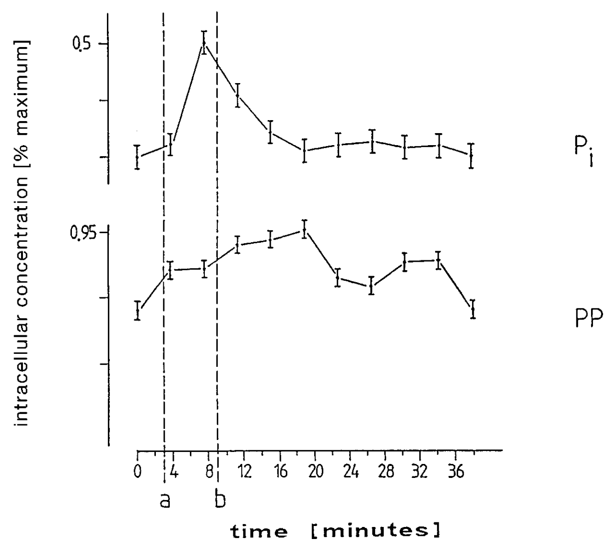

Fig. 1 Intracellular concentrations (as % maximum) of inorganic phosphate (*P_i*) and mobile polyphosphate (*PP*) of the mycelium of *Suillus bovinus* as a function of time in the presence of an external phosphate supply at a constant pH of 3.5. The supply period of Hoagland solution containing 0.1 mM KH_2PO_4 is marked by the intervall *a–b.* Each data point represents the integral of the peak taken in arbitrary units from in vivo ^{31}P-NMR spectra of 1600 scans each

8.5 (data not shown). This result verifies the constancy in internal pH of roots and fungus during the experiments.

Uptake and storage

The uptake and storage of phosphate was influenced by the external pH. The viability of the objects within the NMR tube was limited to 28–30 h, which allowed only relatively brief periods of accumulation and therefore small numbers of added scans at the NMR spectrometer. For Figures 2–4, the upper part (a) shows the affects on uptake and storage in the range of external pH 6.5–3.5, returning to pH 6.5, and the lower part (6) in the range 6.5–8.5, returning to pH 6.5 (Figs. 2b–4b). The direction of change in pH is indicated by arrows. Values for P_i and PP at each pH are expressed as percent of the absolute maximum recorded.

With external pH in the lower range, the pure mycelium of *S. bovinus* had maximal phosphate uptake at pH 5.5, and a minimum at pH 3.5 (Fig. 2a). On returning to pH 6.5, the phosphate uptake reached a lower value than at the start. With completion of the pH cycle 6.5–3.5–6.5, the polyphosphate concentration dropped to approximately 85%. In contrast to the results obtained with *S. bovinus,* no variation in phosphate uptake was found in the nonmycorrhizal roots of *P. sylvestris* within this same pH sequence (Fig. 3a). The mycorrhizal roots of *P. sylvestris* showed maximal P_i uptake at pH 3.5 (Fig. 4a) and a minimum at pH 5.5, i.e. the converse of *S. bovinus*. Likewise, in contrast to the fungus, PP concentration increased continuously.

In the upper range of external pH, phosphate uptake and storage strongly depended on external pH (Figs. 2b, 3b). As a consequence of the insolubility of phosphate at pH 8.5, uptake of phosphate was not possible at this pH. Nevertheless, with a change from pH 8.5 to 7.5, fungus, nonmycrrhizal and mycorrhizal roots resumed phosphate uptake. For mycelium of *S. bovinus,* the upper pH range yielded extremely variable results for phosphate uptake. The maximal uptake was ca. 62% of maximum and an increase in pH caused a gradual decrease in uptake to zero at 8.5 (Fig. 2b). Subsequently, lowering the external pH from 8.5 to 6.5, caused an increase in phosphate uptake to the maximum at 7.5; this decreased slightly at pH 6.5. In both upper and lower pH ranges, the polyphosphate concentration indicated a consequent dropped (Fig. 2b). In the case of nonmycorrhizal roots, the behaviour of P_i uptake was found to be very simple (Fig. 3b), since the responses to pH were reversible. PP did not occur in nonmycorrhizal roots (see also Gerlitz and Werk 1994). Mycorrhizal roots showed a very similiar behaviour to that of nonmycorrhizal roots with regard to the uptake and storage of phosphate in the pH cycle 6.5–8.5–6.5. The rate of phosphate uptake was lower at pH 6.5 at the end of the experiment than at the beginning (Fig. 4b). In contrast, the PP concentration of mycorrhizal roots increased from pH 6.5 to a maximum at pH 7.5, slightly decreased at pH 8.5, and when roots were returned back to pH 6.5, it finally increased to a higher level than at the start (Fig. 4b).

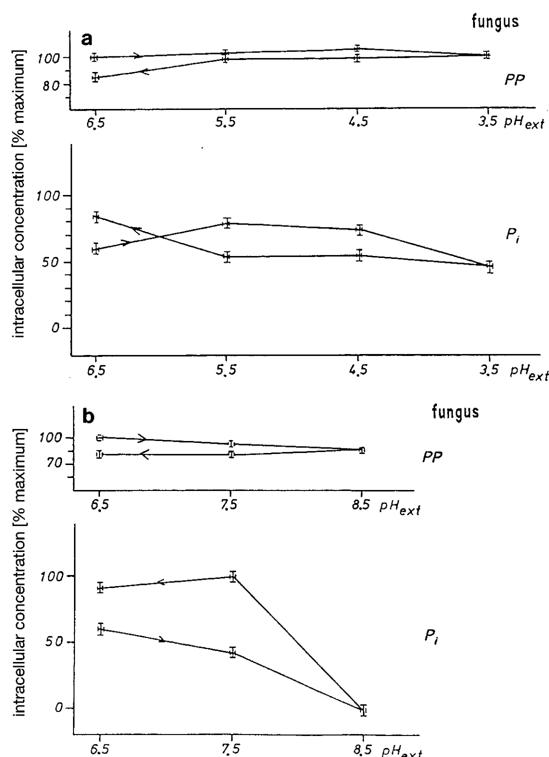

Fig. 2 Uptake of polyphosphate (P_i) and intracellular concentration of mobile polyphosphate (*PP*) of *Suillus bovinus* (expressed as % maximum) as a function of external pH (pH_{ext}) and shown as hysteresis-shaped loops starting and ending at pH 6.5. The direction of external pH variation is marked by arrows. Each data point represents the integral of the ^{31}P-NMR peak, taken in arbitrary units from in vivo ^{31}P-NMR spectra of 1600 scans each

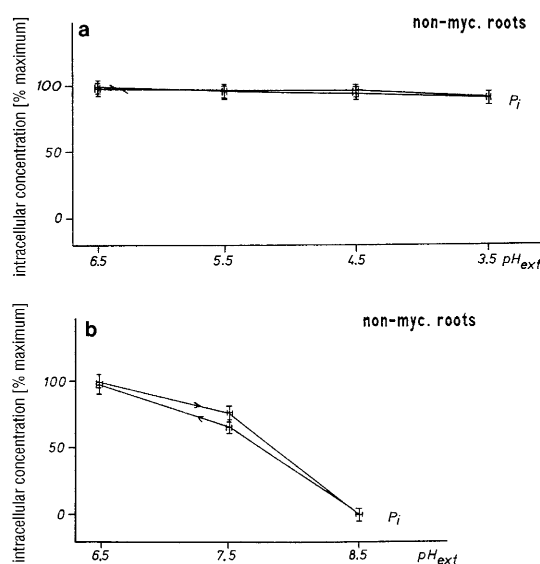

Fig. 3 Same parameters as in Fig. 2 for excised nonmycorrhizal roots of *Pinus sylvestris*

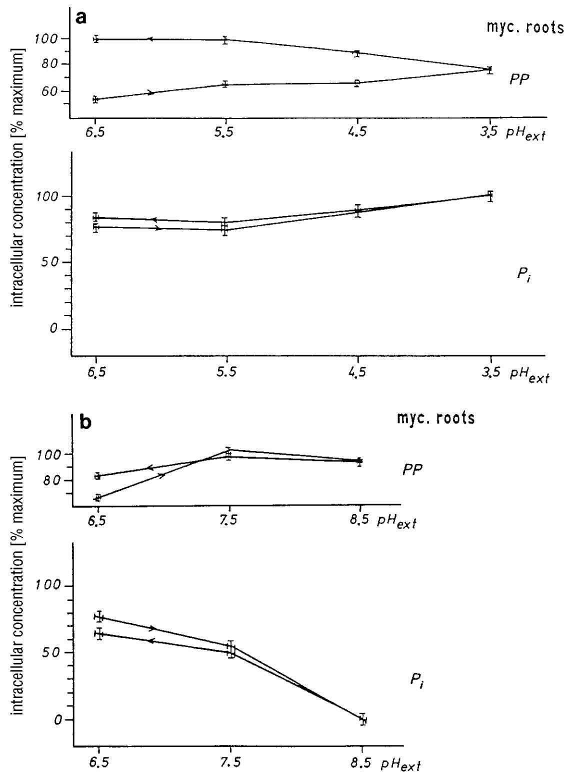

Fig. 4 Same parameters as in Fig. 2 for excised mycorrhizal roots of *Pinus sylvestris*

Discussion

Similiar ^{31}P-NMR investigations to those described here on the dependence of internal pH upon external pH have been previously carried out on mung bean root tips (Torimitsu et al. 1984). In the latter study, a rise in cytoplasmic pH in roots tip of 6.75 to 7.5 was found during an external pH change from 3 to 10. Stability in cytoplasmic pH was found with an external pH range of 4–9. In contrast, with changes in the external pH in the range 3–10, the vacuolar pH of the roots strongly increased from pH 4.4 to 7.4, whereas an increase in external pH from 4 to 9 only produced a rise of 0.5 pH units.

In the present investigation, the intracellular pH of *S. bovinus* and nonmycorrhizal and mycorrhizal roots of *P. sylvestris* remained constant throughout the experiments within an external pH range of 3.5–8.5. A possible reason for this may be found in the three mechanisms proposed by Roos and Boron (1981). The first is a purely passive ion inflow and efflux, the second is assumed to be an active mechanism driven by a Na-K pump, and the third is a model of an energy consumptive, Na-coupled, Cl-HCO_3 exchange. A passive mechanism cannot keep internal pH sufficiently constant for 3–4 h at external pHs of 3.5 and 8.5 and, consequently, an active mechanism requiring ATP must be involved in *S. bovinus* mycelium and both mycorrhizal and nonmycorrhizal roots of *P. sylvestris.* In higher plants, one of these mechanisms is metabolically controlled using malate dehydrogenase/phosphoenolpyruvate carboxylase (Davies 1973). In addition, intracellular pH is maintained by proton transport (Serrano 1984) and cotransport of anions (Hager and Helmle 1981; Hager and Biber 1984; Sze 1985), by an H^+-ATPase (Rea and Poole 1986) and a pyrophosphatase (Hager et al. 1986; Shimmen and MacRobbie 1987). In the present studies, the constant intracellular pH may, therefore, depend on the energy capacity of the samples investigated. In roots, a pool of energy is commonly represented by sugar phosphates. It has been postulated for mycorrhizal fungi that vacuolar polyphosphate could be an energy source, made available by breaking of the energy-rich chemical bonds of the chains of mobile polyphosphate (Harley et al. 1954, 1956; Harley and McCready 1981a; Martin et al. 1985).

All the samples used in the present study were juvenile, i.e the fungus was taken at 3–4 weeks growth and excised roots were sampled 2 months after inoculation of *P. sylvestris* seedlings with *S. bovinus* (Cairney and Alexander 1992; Downes et al. 1992). Phosphate uptake by mycorrhizal and nonmycorrhizal roots of *P. sylvestris* and by *S. bovinus* showed smooth kinetics in the cycle pH 6.5–3.5–6.5. The two symbionts alone had a maximal rate of phosphate uptake at an external pH of ca. 6.5, whereas in mycorrhizas the maximum depended on the direction of the external pH shift.

Nonmycorrhizal roots did not contain polyphosphate at any pH but mycorrhizal roots showed an increase in polyphosphate concentration with a change in external pH from 6.5 to 3.5; reveral of the pH change produced a stabilization at pH 5.5. Transformation of intracellular inorganic phosphate into the storage form of polyphosphate is advantageous for further uptake of phosphate by lowering the cellular pools of inorganic phosphate (Gerlitz and Werk 1994). However, a distinction has to be made between so-called mobile and immobile polyphosphates (Gerlitz and Werk 1994). The first form has a middle chain-length up to ca. 100 ($n \leq 100$) and is, therefore, detectable by in vivo ^{31}P-NMR. In contrast, the second form has a long chain-length ($n \gg 100$) and a granular form and is not detectable by this technique (Gadian 1982). Therefore, an increase or decrease in mobile polyphosphate concentration could be caused by transformation from or into immobile polyphosphate as well as inorganic phosphate (P_i). Thus polyphosphate formation by fungal or mycorrhizal tissues may be important both for storing energy in the form of the high energy chemical bonds of polyphosphate chains (Harley 1978a, b; Harley and McCready 1981b) and for maintaining the phosphorus concentration within fungal cells (MacFall et al. 1992; Martin et al. 1985; Mousain and Salsac 1985).

Since transformation of P_i into mobile polyphosphate and from mobile into immobile polyphosphate occurs within 38 min (Gerlitz and Werk 1994), any deficiency in fungal and mycorrhizal P_i caused by the exter-

nal pH should be detectable by changes in PP concentration within the fungal cells. During the external pH change sequence 6.5–8.5–6.5, the rate of phosphate uptake by *S. bovinus* after the change 6.5 to 8.5 differed from that after 8.5 to 6.5, in contrast to the results with roots, suggesting that the fungus alone reacts more slowly to an external pH change than roots. Above an external pH of 6.5, fungal cells seemed to need time to adapt to a higher pH since an increase in phosphate uptake occurred when the pH was reversed to 6.5. A simiiliar process occurred with a change in the external pH of 6.5–3.5–6.5. In addition, at pH 8.5 there was no detectable uptake of phosphate. This may be caused by an increase in insoluble $Ca(HPO_4)$ in the Hoagland's solution, and thus a decrease in available phosphate with an increase in pH. As a consequence, there was a deficiency in phosphate for fungal metabolism. On returning to pH 6.5, the availability of phosphate would induce the fungus to refill pools of both inorganic phosphate and polyphosphate, and this is seen in an increased phosphate uptake.

The excised nonmycorrhizal and mycorrhizal roots followed the same trend for P_i. Although both showed a more moderate reaction to external pH than *S. bovinus* in the upper pH range with regards to P_i uptake, the latter decreased in nonmycorrhizal and mycorrhizal roots with an increase in pH, and P_i uptake was no longer detectable at pH 8.5. Since roots do not contain polyphosphates, they will mainly refill the P_i pools with a return to pH 6.5 so that P_i uptake will be related to external pH. In contrast, an important mobilization of PP was evident in mycorrhizal roots with shifts in external pH, while the concentration of mobile PP in the *S. bovinus* decreased continually. Mobilization of PP in mycorrhiza in the pH range 7.5–8.5 indicates a stress response (Gerlitz and Werk 1994), whilst the pure fungal mycelium seems to be protected against the effects of higher pHs. The mobilization of PP in the mycorrhiza may be related to functioning of the symbiotic interface localized between the symbiotic root and fungal cells in the Hartig Net (Clarkson 1985; Kottke and Oberwinkler 1987; Smith and Smith 1986, 1989; Woolhouse 1975). Although the pH of the interface has not been determined, it is likely that a weak acid pH is maintained there due to active H^+-ATPases in the bordering plant and fungal plasma membranes (Smith and Smith 1989, 1990). It can be speculated that if a strong alkaline solution penetrates into the interface of the Hartig Net from the external medium, interfacial pH will rise. Plasma membrane H^+-ATPases will continually act against the increased concentration of OH^- ions in order to maintain an intracellular acid pH in both symbionts. This higher energy requirement may be met by the intracellular store of fungal PP.

References

Cairney JWG, Alexander IJ (1992) A study of ageing of spruce [*Picea sitchensis* (Bong.) Carr.] ectomycorrhizas. III. Phosphate absorption and transfer in ageing *Picea sitchensis*/*Tylospora fabrillosa* (Burt.) Donk ectomycorrhizas. New Phytol 122:159–164

Cairney JWG, Jennings DH, Ratcliffe RG, Southern TE (1988) The physiology of basidiomycetes linear organs. II. Phosphate uptake by rhizomorphs of *Armillaria mellea.* New Phytol 109:327–333

Clarkson DT (1985) Factors affecting mineral nutrient acquisition by plants. Annu Rev Plant Physiol 38:77–115

Colpaert JV, Van Assche P (1992) Zinc toxicity in ectomycorrhizal *Pinus sylvestris.* Plant Soil 143:201–211

Davies DD (1973) Metabolic control in higher plants. In: Milborrow BV (ed) Biosynthesis and its control in plants. Academic Press, London, pp 1–20

Downes GM, Alexander IJ, Cairney JWG (1992) A study of ageing of spruce [*Picea sitchensis* (Bong.) Carr.] ectomycorrhizas. I. Morphological and cellular changes in mycorrhizas formed by *Tylospora fabrillosa* (Burt.) Donk and *Paxillus involutus* (Batsch. Ex Fr.) New Phytol 122:141–152

Finlay RD, Read DJ (1986) The structure and function of the vegetative mycelium of ectomycorrhizal plants. II. The uptake and distribution of phosphorus by mycelial strands interconnecting host plants. New Phytol 103:157–185

Gadian DG (1982) Nuclear magnetic resonance and its applications to living systems. Oxford University Press, New York

Gerlitz TGM, Werk WB (1994) Investigations on phosphate uptake and polyphosphate metabolism by mycorrhized and nonmycorrhized roots of beech and pine as investigated by in vivo ^{31}P NMR. Mycorrhiza 4:207–214

Grellier B, Strullu DG, Martin F, Renaudin S (1989) Synthesis in vitro, microanalysis and ^{31}P-NMR study of metachromatic granules in birch mycorrhizas. New Phytol 112:49–54

Hager A, Biber W (1984) Functional and regulatory properties of H^+ pumps at the tonoplast and plasma membranes of *Zea mays* coleoptiles. Z Naturforsch 39:927–937

Hager A, Helmle M (1981) Properties of an ATP-fueled, Cl^--dependent proton pump localized in membranes of microsomal vesicles from maize coleoptiles. Z Naturforsch 36:997–1008

Hager A, Berthold W, Biber W, Edel H-G, Lanz C, Schiebel G (1986) Primary and secondary energized ion translocating systems on membranes of plant cells. Ber Dtsch Bot Ges 99:281–295

Harley JL (1978a) Ectomycorrhizas as nutrient absorbing organs. Proc R Soc London B 203:1–21

Harley JL (1978b) Nutrient absorption by ectomycorrhizas. Physiol Vég 16:543–555

Harley JL, McCready CC (1981a) Phosphate accumulation in *Fagus* mycorrhizas. New Phytol 89:75–80

Harley JL, McCready CC (1981b) The relationship between phosphate absorption and oxygen uptake in excised beech mycorrhizas. New Phytol 88:675–681

Harley JL, Smith SE (1983) Mycorrhizal symbiosis. Academic Press, London

Harley JL, Brierly JK, McCready CC (1954) The uptake of phosphate by excised mycorrhizal roots of the beech. V. The examination of possible sources of misinterpretation of the quantities of phosphorus passing into the host. New Phytol 53:92–98

Harley JL, McCready CC, Brierly JK, Jennings DH (1956) The salt respiration of excised beech mycorrhizas. II. The relationship between oxygen consumption and phosphate absorption. New Phytol 55:1–28

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. Circular Calif Agric Exp Stn 347

Kottke I, Oberwinkler S (1987) The cellular structure of the Hartig net: coenocytic and transfer cell-like organisation. Nord J Bot 7:85–95

Kottke I, Guttenberger M, Hampp R, Oberwinkler S (1987) An in vitro method for establishing mycorrhizal on coniferous tree seedlings. Trees 1:191–194

Lee RB, Ratcliffe RG (1983) Phosphorus nutrition and the intracellular distribution of inorganic phosphate in pea root tips: a quantitative study using ^{31}P NMR. J Exp Bot 34:1222–1244

MacFall JS, Slack SA, Wehrli S (1992) Phosphorus distribution in red pine roots and the ectomycorrhizal fungus *Hebeloma arenosa.* Plant Physiol 100:713–717

Manion PD (1981) Tree disease concepts. Prentice Hall, Englewood Cliffs, NJ

Martin F, Marchal JP, Tyminska A, Canet D (1985) The metabolism and physical state of polyphosphates in ectomycorrhizal fungi. A ^{31}P nuclear magnetic resonance study. New Phytol 101:275–290

Marx DH, Bryan WC (1975) Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infested with the fungal symbiont *Pisolithus tinctorius.* For Sci 21:245–254

Mousain D, Salsac L (1985) Growth and accumulation of phosphorus in the ectomycorrhizal fungus *Pisolithus tinctorius.* In: Gianinazzi-Pearson V, Gianinazzi S (eds) Mycorrhizae: physiology and genetics. INRA, Paris, pp 357–361

Pfeffer PE, Tu S-I, Gerasimowicz WV, Boswell RT (1987) Effects of aluminium on the release and/or immobilization of soluble phosphate in corn root tissue. A ^{31}P-nuclear magnetic resonance study. Planta 172:200–208

Rea PA, Poole RJ (1986) Chromatographic resolution of H^+-translocating pyrophosphate from H^+-translocating ATPase of higher plant tonoplasts. Plant Physiol 81:126–129

Roberts JKM, Ray PM, Wade-Jardetzky N, Jardetzky O (1980) Estimation of cytoplasmic and vacuolar pH in higher plant cells by ^{31}P NMR. Nature London 283:870–872

Roberts JKM, Wade-Jardetzky N, Jardetzky O (1981) Intracellular pH measurements by ^{31}P-nuclear magnetic resonance. Influence of factors other than pH on ^{31}P chemical shifts. Biochemistry 20:5389–5394

Roos A, Boron WF (1981) Intracellular pH. Physiol Rev 61:296–434

Serrano R (1984) Plasma membrane ATPase of fungi and plants as a novel type of proton pump. Curr Top Cell Regul 23:87–126

Shimmen T, MacRobbie EAC (1987) Demonstration of two proton translocating systems in tonoplasts of permabilized *Nitella* cells. Protoplasma 136:205–207

Smith FA, Smith SE (1986) Movement across membranes: physiology and biochemistry. In: Gianinazzi-Pearson V, Gianinazzi S (eds) Physiological and genetical aspects of mycorrhizae. INRA, Paris, pp 75–84

Smith FA, Smith SE (1989) Membrane transport at the biotrophic interface: an overview. Aust J Plant Physiol 16:33–43

Smith SE, Smith FA (1990) Structure and function of the interfaces in biotrophic symbiosis as they relate to nutrient transport. New Phytol 114:1–38

Sze H (1985) H^+-translocating ATPases: advances using membrane vesicles. Annu Rev Plant Physiol 36:175–208

Torimitsu K, Yazaki Y, Nagasuka K, Ohta E, Sakata M (1984) Effect of external pH on the cytoplasmic and vacuolar pHs in mung bean root-tip cells: A ^{31}P nuclear magnetic resonance study. Plant Cell Physiol 25:1403–1409

Turnau K, Kozlowska A (1991) Influence of cadmium dust on heavy metal concentration in macromycetes. Zeszyty naukowe uniwersytety Jagiellonskiego, Prace Botaniczne 22:135–141

Turnau K, Kottke I, Oberwinkler F (1993) *Paxillus involutus–Pinus sylvestris* mycorrhizae from heavily polluted forest. I. Element localization using electron energy spectroscopy and imaging. Bot Acta 106:213–219

Woolhouse HW (1975) Membrane structure and transport problems considered in relation to phosphorus and carbohydrate movement and the regulation of endotrophic mycorrhizal associations. In: Sanders FE, Mosse B, Tinker PB (eds) Endomycorrhizas. Academic Press, London, pp 209–239