Phosphate-limitation physiology in ectomycorrhizal pitch pine 
(Pinus rigida) seedlings

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Summary  Foliar and root P concentrations, net H₂PO₄⁻ (Pᵢ) uptake rates, and root surface acid phosphatase (APase) rates were assessed in pitch pine (Pinus rigida Mill.) seedlings inoculated with the ectomycorrhizal fungi Laccaria bicolor (Maire) Pat., Paxillus involutus (Batsch.) Fr., or Pisolithus tinctorius (Pers.) Coker and Couch, and grown at 10 or 100 µM Pᵢ in sand culture. Following a 6-week period of acclimation to the Pᵢ regimes, seedlings grown at 100 µM Pᵢ had greater foliar and root P concentrations than seedlings grown at 10 µM Pᵢ. Mycorrhizal colonization increased the concentration of P in roots and, under Pᵢ-limiting conditions, this retention was at the expense of P translocation to foliage. There were no differences in Pᵢ uptake rates between non-mycorrhizal and mycorrhizal roots grown at 100 µM Pᵢ. However, mycorrhizal colonization enhanced Pᵢ uptake in seedlings grown at 10 µM Pᵢ, with rates 1.3-, 2.6-, and 3.3-fold greater in roots colonized with L. bicolor, P. involutus, and P. tinctorius, respectively, than in non-mycorrhizal roots. Root acid phosphatase (APase) activity was greater in non-mycorrhizal roots than in roots colonized with any of the three mycorrhizal fungi, and increases in activity in response to Pᵢ limitation occurred only in non-mycorrhizal roots. These results highlight the importance of seedling acclimation to prevailing Pᵢ availability and the role of mycorrhizal fungi in altering the allocation of P between roots and shoots. The activities of the APase systems of the mycorrhizal species tested do not support the hypothesis that this enzyme system plays an important role in Pᵢ acquisition under Pᵢ-limiting conditions.

Keywords: acclimation, acid phosphatase, ectomycorrhizal fungi, phosphorus uptake.

Introduction

Phosphorus availability and uptake by plants is often limited by the diffusion of H₂PO₄⁻ (Pᵢ) to plant roots (Dalal 1977). The concentration of Pᵢ in the bulk soil solution is, in turn, depend-ent on the distribution of P between soluble and insoluble inorganic and organic complexes (Dalal 1977). Although the proportions of Pᵢ and organic P may differ among soils and environmental conditions, a majority of the total P pool is normally found in organic forms (Harrison 1983, Fernandez and Struchemeyer 1985, Condron et al. 1990). In response to the limited availability of Pᵢ in soils and its distribution in complex forms, plants have developed metabolic systems and ecological relationships both to absorb Pᵢ, and to access complex P-containing compounds in the rhizosphere.

Plants grown under Pᵢ-limiting conditions exhibit several physiological changes that reflect the activity of metabolic systems triggered by P deprivation. Phosphate starvation leads to coordinated changes in patterns of growth, de novo production and secretion of P-scavenging enzymes, derepression of P transporters, and possibly de novo production of P transporters with higher affinities for Pᵢ (Ninomiya et al. 1977, Ueki 1978, Clarkson and Scattergood 1982, Goldstein et al. 1988, 1989). The induction of such systems may play a crucial role in increasing Pᵢ availability in soils and Pᵢ uptake by roots (Clarkson and Luttege 1991, Duff et al. 1994).

In woody species, the association of ectomycorrhizal fungi with roots often increases host plant acquisition of Pᵢ from soils (Harley and Smith 1983). This may be the result of increased short root length induced by mycorrhizal colonization or increased rhizomorph production by the fungi. These morphological changes increase the volume of soil exploited by mycorrhizal plants and reduce diffusive limitations to Pᵢ availability (Bolan 1991). Additionally, ectomycorrhizal fungi may increase Pᵢ acquisition through the activity of Pᵢ transporters that have high affinities for Pᵢ or that function at low threshold Pᵢ concentrations (Bolan 1991, Clarkson and Luttege 1991). These changes in root morphology and physiology are most pronounced when Pᵢ is limiting to plant function.

In addition to increasing Pᵢ uptake, physiological changes in the root resulting from mycorrhizal colonization may lead to enhanced acid phosphatase (APase) activity, causing Pᵢ to be scavenged from organic pools (Bartlett and Lewis 1973, Williamson and Alexander 1975). Several authors have noted that APase activities of ectomycorrhizae increase in response to Pᵢ deprivation, leading to the hypothesis that mycorrhizae are responsible for effective P utilization by trees growing under Pᵢ-limiting conditions (Ho and Zak 1979, Antibus et al. 1986, Kroehler et al. 1988, MacFall et al. 1991).

The association of Pisolithus tinctorius with roots of pitch pine seedlings has been shown to increase seedling growth and P accumulation under Pᵢ-limiting conditions (Cumming and Weinstein 1990, Cumming 1993). The goal of the present work was to test the ability of mycobionts other than P. tinctorius to
acquire P\textsubscript{i} under P\textsubscript{i}-limiting conditions. I assessed two P\textsubscript{i} limitation-induced physiological systems that may function to circumvent P\textsubscript{i} limitation in pitch pine: (1) seedling P nutrient status and trans-membrane P\textsubscript{i} uptake rates, and (2) extracellular APase activities.

Materials and methods

Pitch pine (Pinus rigida Mill.) seedlings were propagated in a sand-hydroponic culture system, as described by Cumming and Weinstein (1990). Seeds were surface-sterilized in 30% H\textsubscript{2}O\textsubscript{2} for 30 min and germinated in tall petri dishes containing perlite. Germinating seeds and seedlings were watered daily with 0.25-strength modified Johnson’s solution (Johnson et al. 1957 as modified by Cumming and Weinstein 1990), consisting of 1.5 mM KNO\textsubscript{3}, 1.0 mM Ca(NO\textsubscript{3})\textsubscript{2}-H\textsubscript{2}O, 0.1 mM (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, 0.1 mM NH\textsubscript{4}H\textsubscript{2}PO\textsubscript{4}, 0.25 mM MgSO\textsubscript{4}-6H\textsubscript{2}O, 20 µM Fe-EDTA, 50 µM KCl, 25 µM H\textsubscript{2}BO\textsubscript{3}, 2.0 µM MnSO\textsubscript{4}-H\textsubscript{2}O, 0.20 µM ZnSO\textsubscript{4}-7H\textsubscript{2}O, 0.5 µM CuSO\textsubscript{4}-5H\textsubscript{2}O, and 0.5 µM Na\textsubscript{2}MoO\textsubscript{4}. All solutions were adjusted to pH 4.5. Seedlings were maintained in a growth chamber providing a day/night temperature of 24/19 °C, a 14-h photoperiod, and photosynthetically active radiation of 500 µmol m\textsuperscript{−2} s\textsuperscript{−1} from a combination of fluorescent and incandescent sources. Relative humidity was not controlled but was maintained at high values by placing water-filled pans in the bottom of the growth chamber.

Fungal inoculum was prepared by inoculating 20 ml of liquid Modified Melin-Norkrans (MMN) medium (pH 4.5) (Molina and Palmer 1982) with two 5-mm hyphal plugs taken from the edges of 3-week-old cultures of Laccaria bicolor (Maire) Pat., Paxillus involutus (Batsch.) Fr., or Pisolithus tinctorius (Pers.) Coker and Couch grown on MMN agar. Inoculum was grown in tissue culture vessels (Sigma Chemical Company, St. Louis, MO) for three weeks at 20 °C before inoculation of pitch pine seedlings.

Following three weeks of growth in perlite, pitch pine seedlings were transplanted to Stuewe D16 containers (Stuewe and Sons, Corvallis, OR) containing 350 cm\textsuperscript{3} of acid-washed silica sand. At the time of transfer, seedling root tips were excised and seedlings were inoculated with liquid inoculum of one of the three mycobionts. Liquid inoculum was prepared by rinsing fungal cultures in distilled H\textsubscript{2}O and blending hyphae twice for 5 s each time. Two ml (approximately 10 mg dry weight) of inoculum was placed in close proximity to the root system. Non-inoculated seedlings were also established. Seedlings were watered twice daily with 25 ml of 0.25-strength modified Johnson’s solution.

Following two weeks of growth in sand culture, nutrient solutions were altered to deliver either 10 or 100 µM P, in a factorial experimental design with the three inoculation treatments. The salts NH\textsubscript{4}H\textsubscript{2}PO\textsubscript{4} and (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} were used to deliver a variable P concentration with a constant NH\textsubscript{4} concentration. These P concentrations were considered limiting (10 µM) or sufficient (100 µM), based on previous experiments (Cumming and Weinstein 1990). Seedlings were grown in the P\textsubscript{i} treatments for six weeks. At the time of physiological measurements, root systems of mycorrhizal seedlings exhibited > 75% colonization of short roots in all fungal treatments (visual assessment of intact root systems), and there was no evidence of cross contamination among the mycobionts studied. Seedlings for which inoculation was ineffective were not included in experimental analyses.

Root physiological responses to fungal colonization and P availability were studied by transferring seedlings from sand culture to hydroponic culture. The hydroponic systems consisted of 2-liter plastic containers covered with aluminum foil and black tape to exclude light. Solutions were vigorously aerated. One seedling of each fungal treatment and two non-mycorrhizal seedlings were transferred as a group to a given culture vessel containing nutrient solution at the same P concentration as the sand culture in which they were propagated. There was no visible evidence of mycorrhizal cross-contamination with other mycorrhizal treatments even though seedlings with different mycobionts shared the same hydroponic system.

Although root colonization may occur rapidly when inoculum is vigorously growing on glucose-amended substrate (Fortin and Piche 1980), colonization is much slower when exogenous carbon is not supplied (Duddridge and Read 1984).

At the time of transfer from sand to hydroponic culture, two third-order lateral roots with abundant short roots were excised and transferred to 9 ml of the appropriate growth nutrient solution in 50-ml centrifuge tubes. These roots were incubated with aeration at 20 °C for 30 min. Root acid phosphatase (APase) activity was determined by the hydrolysis of p-nitrophenylphosphate (NPP) over a 30-min period, according to a modified method of Tabatabai and Bremner (1969) as described by Cumming (1993). The production of nitrophenol (NP) was adjusted for time and root fresh weight.

Nutrient solutions in the hydroponic systems were changed every 12 h for 72 h before the assessment of P\textsubscript{i} uptake. The net uptake of \(^{32}\text{P}\) was measured on intact seedlings in hydroponic solutions containing either 10 or 100 µM P. The experiment was designed so that uptake P\textsubscript{i} concentrations were crossed with propagation P\textsubscript{i} concentrations such that \(^{32}\text{P}\) uptake was measured in seedlings propagated at 10 and 100 µM P\textsubscript{i} in solutions containing either 10 or 100 µM P\textsubscript{i} (Figure 1).

Seedlings were acclimated to uptake solutions for 30 min before KH\textsubscript{2}PO\textsubscript{4} was added to give a specific activity of approximately 2500 cpm (µmol P\textsubscript{i})\textsuperscript{−1}. Seedlings were exposed to labeled solutions for 20 min. Following uptake, seedling root systems were rinsed in ice-cold distilled H\textsubscript{2}O for 5 s, and desorbed in ice-cold nutrient solution containing 100 µM P\textsubscript{i} for 4 min, followed by a 1-min rinse in cold 0.5 mM CaSO\textsubscript{4} solution. Root systems were blotted dry and roots and shoots were separated. Two sets of two third-order lateral roots were excised from the root system, weighed in tared scintillation vials, and cleared by boiling in 2 ml of a solution consisting of 70% 1.25 M NaOH and 30% household bleach. Five ml of distilled H\textsubscript{2}O was added to each vial and \(^{32}\text{P}\) in root segments was determined by Cerenkov radiation spectrometry. Root cpm data were normalized to uptake solution specific activity and root fresh weight (g\textsubscript{fw}), and analyzed as µmol P g\textsubscript{fw}\textsuperscript{−1} h\textsuperscript{−1}.

For comparison with other studies, the dry weight/fresh weight
ratios of roots in the present study were approximately 0.1 across all treatments.

To assess the short-term translocation of P to foliage, two sets of three primary needles were excised from the mid-portion of each seedling shoot following seedling exposure to $^{32}\text{P}_i$, chopped into 2-mm segments and weighed in tared scintillation vials. The needle samples were cleared and radioactivity determined as described above. Needle cpm data were normalized to uptake solution specific activity and root fresh weight, and analyzed as nmol P g$^{-1}$ fw h$^{-1}$.

Seedlings were grown in a blocked factorial arrangement to account for environmental variability within the growth chamber. Seedlings were harvested by block over time, such that the block effect is confounded with the replicate effect. Inoculation treatments were replicated once per block; non-inoculated treatments were replicated twice per block. One complete block (inoculation by propagation $P_i$ by uptake $P_i$) was represented by 20 seedlings. Uptake and APase data were analyzed using the statistical package JMP (SAS Institute, Inc., Cary, NC). Data were subjected to analysis of variance using a blocked factorial model. Single degree-of-freedom contrasts were used to compare means within effects. All means presented are least square means and are accompanied by standard errors of the least square means.

Results

Seedling nutrition

Both foliar and root P concentrations were influenced by growth [$P_i$] and fungal colonization, with significant interactions between these treatments (Figure 2). Across all inoculation treatments, foliar P concentrations more than doubled when seedlings were grown at 100 µM $P_i$, compared with seedlings grown at 10 µM $P_i$ (Figure 2). Among seedlings grown at 10 µM $P_i$, however, foliar P concentrations of seedlings colonized with $L.\ bicolor$ and $P.\ tinctorius$ were 37 and 29% lower than those of non-inoculated controls ($P = 0.003$ and 0.013 for the single degree of freedom contrasts, respectively).

Colonization of roots by all three mycobionts increased root P concentrations above that of non-mycorrhizal controls at both 10 and 100 µM $P_i$ (Figure 2). The increases were most pronounced for seedlings colonized with $P.\ tinctorius$, where root P concentrations were 1.6- and 1.8-fold greater in seedlings grown at 10 and 100 µM $P_i$, respectively ($P < 0.001$ for the differences in each case), than in the corresponding non-mycorrhizal seedlings. These patterns indicate that the mycobionts were strong sinks for P and that this sink strength altered seedling P allocation under $P_i$-limiting conditions.

$^{32}P$ Fluxes

The shift in P allocation from shoots to roots was also apparent in short-term $^{32}P$ translocation rates from roots to shoots (Table 1). Colonization of root systems by mycorrhal fungi depressed the transport of newly absorbed P from roots to shoots by up to 62 and 97% in seedlings grown with 100 and 10 µM $P_i$, respectively, compared with non-mycorrhizal controls (Table 1). Phosphorus translocation rate was elevated 4-fold by $P_i$ limitation in non-mycorrhizal seedlings, whereas this increase was much less pronounced or absent in mycorrhizal seedlings (Table 1).
When seedlings were grown with 100 \( \mu \text{M P} \), there were no significant differences between non-mycorrhizal and mycorrhizal treatments in net \( \text{P}_\text{i} \) uptake by roots (Figure 3). Seedlings grown under \( \text{P}_\text{i} \)-limiting conditions exhibited enhanced \( \text{P}_\text{i} \) uptake rates compared with seedlings grown with 100 \( \mu \text{M P} \), although the extent of the stimulation was dependent on the mycobiont (\( P < 0.001 \) for the fungus main effect for seedlings grown at 10 \( \mu \text{M P} \)) (Figure 3). Uptake rates by roots colonized with \textit{L. bicolor}, \textit{P. involutus}, and \textit{P. tinctorius} were 1.56, 2.36, and 3.11 times greater (\( P = 0.016, < 0.001, \) and \( < 0.001 \), respectively, for contrasts) than uptake rates of non-mycorrhizal seedlings (Figure 3).

An examination of net \( ^{32}\text{P} \) uptake as a function of \( \text{P}_\text{i} \) concentration during growth and uptake indicated that the mycorrhizal fungi did not influence transport characteristics of roots when grown with sufficient \( \text{P}_\text{i} \) (\( P = 0.266 \) for the fungus by uptake \( \text{P}_\text{i} \) interaction) (Figure 4, lower panel). In contrast, when seedlings were grown under \( \text{P}_\text{i} \)-limiting conditions and exposed to uptake solutions containing either 10 or 100 \( \mu \text{M \( ^{32}\text{P} \))}, there were significant differences between inoculation treatments that were dependent on uptake \( ^{32}\text{P} \), concentration (\( P < 0.001 \) for the fungus by uptake \( \text{P}_\text{i} \) interaction). This interaction was driven by the varying magnitude of change in \( ^{32}\text{P} \) uptake between the 10 and 100 \( \mu \text{M \( ^{32}\text{P} \) regimes for the different mycorrhizal treatments (Figure 4, top panel).}

Because seedlings were propagated in flow-through nutrient culture and the rates of \( ^{32}\text{P} \) uptake were measured in well-mixed hydroponic solutions, differences in net uptake rates reflect differences in transport physiology and not an alteration of diffusive resistances to uptake conferred by root colonization. The larger surface area of colonized roots, which increased the surface area for absorption, also led to significantly greater short root unit mass. Thus, \( \text{P}_\text{i} \) flux measures normalized to root mass will account for a significant portion of this mycobiont-enhanced surface area.

The basis for the observed differences among inoculation treatments was explored by analyzing two ratios calculated from the uptake data (Table 2). The steady-state ratio reflects the degree of acclimation to \( \text{P}_\text{i} \)-limiting conditions. A ratio of

Table 1. Translocation\(^1\) of \( \text{P}_\text{i} \) (nmol \( \text{P}_\text{i} \text{ g}^{-1} \text{ h}^{-1} \)) to foliage of pitch pine seedlings grown with 10 or 100 \( \mu \text{M P} \). Standard errors are shown in parentheses.

<table>
<thead>
<tr>
<th>Inoculant</th>
<th>Growth [( \text{P}_\text{i} )]</th>
<th>10 ( \mu \text{M} )</th>
<th>100 ( \mu \text{M} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>18.56 (0.75)</td>
<td>4.62 (0.76)</td>
</tr>
<tr>
<td>\textit{L. bicolor}</td>
<td></td>
<td>0.59 (1.07)</td>
<td>2.09 (1.23)</td>
</tr>
<tr>
<td>\textit{P. involutus}</td>
<td></td>
<td>4.60 (1.29)</td>
<td>2.43 (1.18)</td>
</tr>
<tr>
<td>\textit{P. tinctorius}</td>
<td></td>
<td>3.23 (1.07)</td>
<td>1.75 (1.07)</td>
</tr>
</tbody>
</table>

\(^1\) Translocation calculated as total shoot \( ^{32}\text{P} \) normalized to uptake time and root fresh weight; means and standard errors calculated across uptake [\( \text{P}_\text{i} \)] (\( n = 10 \) for mycobionts, \( n = 20 \) for non-inoculated seedlings); \( P < 0.001 \) for the inoculation by growth \( \text{P}_\text{i} \) interaction.

Figure 3. Net \( ^{32}\text{P} \) uptake (nmol \( \text{g}_\text{fw}^{-1} \text{ h}^{-1} \)) by roots of pitch pine seedlings colonized with ectomycorrhizal fungi and grown with 10 or 100 \( \mu \text{M P} \). Values are marginal means across uptake [\( \text{KH}_2^{32}\text{PO}_4 \)] and reflect the main effect of growth \( \text{P}_\text{i} \) concentration on transport characteristics.

Figure 4. Net uptake of \( ^{32}\text{P} \) by roots of pitch pine seedlings colonized with ectomycorrhizal fungi. Seedlings were grown with 10 \( \mu \text{M P} \) (top panel) or 100 \( \mu \text{M P} \) (bottom panel) and were exposed to 10 or 100 \( \mu \text{M \( ^{32}\text{P} \)) in a crossed design. Symbols: (\( \blacksquare \)) non-mycorrhizal; (\( \circ \)) \textit{Laccaria bicolor}; (\( \bigcirc \)) \textit{Paxillus involutus}; (\( \triangle \)) \textit{Pisolithus tinctorius}. Note differences in scale between top and bottom panels.
one indicates physiological adjustment to $P_i$ supply because rates of $P_i$ uptake are equal, regardless of the supply of $^{32}P_i$. Ratios greater than one indicate potential changes in transport physiology that allow enhanced $P_i$ uptake when $P_i$ is limiting. For non-mycorrhizal roots and roots colonized with $L. bicolor$, uptake rates of $P_i$ from solutions containing 10 $\mu M$ $^{32}P_i$ did not differ significantly from $P_i$ uptake rates from solutions containing 100 $\mu M$ $^{32}P_i$, indicating that both acclimated to low $P_i$ availability (Table 2). Roots colonized with $P. involutus$ or $P. tinctorius$ exhibited steady-state ratios significantly greater than one, indicating that roots colonized with these fungi and grown under $P_i$ limitation were more effective than $P_i$-sufficient seedlings at acquiring $P_i$ from the environment.

The affinity ratio reflects the efficiency of uptake at low $P_i$ availability, as influenced by $P_i$ concentration during growth. Ratios greater than one reflect an enhancement of uptake system transport capacity (increased affinity) in response to $P_i$ limitation. Non-mycorrhizal roots and roots colonized with the three fungal symbionts exhibited greater transporter affinity when grown under $P_i$-limiting conditions than under $P_i$-sufficient conditions (Table 2). Roots colonized with $P. involutus$ or $P. tinctorius$ exhibited higher affinity ratios than either non-mycorrhizal roots or roots colonized with $L. bicolor$, indicating that these two fungi were extremely effective at responding to $P_i$ limitation.

In contrast to changes in $P_i$ transport physiology brought about by $P_i$ limitation, root surface APase activity did not change within the fungal inoculation treatments in response to growth $P_i$ concentration ($P = 0.468$ for the growth $[P_i]$ main effect) (Figure 5). Root APase activity of non-mycorrhizal roots increased by 13.7% in response to $P_i$ limitation ($P = 0.046$). The APase rates of roots colonized with $L. bicolor$, $P. involutus$, and $P. tinctorius$ were 37.8, 28.8, and 25.0%, respectively, of the APase rates of non-mycorrhizal roots ($P < 0.001$ for the fungus main effect) (Figure 5).

### Discussion

The supply of $P_i$ from the soil to the plant is often neither constant nor optimal for plant growth. Changes in root physiology in response to changes in soil phosphate concentration are therefore likely to play a crucial role in maintaining the supply of nutrients to plants growing on nutrient-limited substrates (Bolan 1991, Clarkson and Luttge 1991, Duff et al. 1994). Phosphorus deprivation may lead to the production of ion transporters with enhanced affinity for $P_i$, thereby increasing uptake efficiency (Cartwright et al. 1972, Furihata et al. 1992), or may increase $P_i$ uptake capacity by increasing the total number of transporters in the plasma membrane (Clarkson and Scattergood 1982, Drew et al. 1984, Shimogawara and Usada 1995).

In addition to increasing their capacity for $P_i$ uptake, roots may alter chemical equilibria in the rhizosphere and access $P$-containing pools not readily available to the plant. The production of extracellular APases with low substrate specificities may increase the turnover of phosphomonoesters in the rhizosphere, increasing the availability of $P_i$ to the root (Duff et al. 1994). The production of APases under $P_i$-limiting conditions often coincides with increases in $P_i$ uptake capacity (Duff et al. 1981, Lee 1982, Goldstein et al. 1989), suggesting that these two metabolic systems function in concert to enhance $P_i$ availability in the soil and uptake of $P_i$ by the plant.

In the present study, non-mycorrhizal seedlings and seedlings inoculated with the ectomycorrhizal symbiots $L. bicolor, P. involutus$, and $P. tinctorius$ exhibited complex patterns of $P$ nutrition and $P_i$ transport in response to changing $P_i$ availability. When grown with 100 $\mu M$ $P_i$, foliar $P$ concentrations and rates of $^{32}P_i$ uptake by roots were similar among mycorrhizal treatments and differed only in response to $^{32}P_i$ concentration (Figures 3 and 4). Root colonization by all mycohionts altered the allocation of $P$ within the seedling, with significantly greater $P$ retained in mycorrhizal than in non-mycorrhizal roots. When $P_i$ supply was sufficient, this apparent

### Table 2. Steady-state$^1$ and affinity$^2$ ratios for pitch pine seedlings inoculated with ectomycorrhizal fungi.

<table>
<thead>
<tr>
<th>Inoculant</th>
<th>Steady-state ratio</th>
<th>Affinity ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.93</td>
<td>1.88$^b$</td>
</tr>
<tr>
<td>$L. bicolor$</td>
<td>0.88</td>
<td>4.04$^b$</td>
</tr>
<tr>
<td>$P. involutus$</td>
<td>1.84$^{a,b}$</td>
<td>7.07$^{a,b}$</td>
</tr>
<tr>
<td>$P. tinctorius$</td>
<td>1.82$^{a,b}$</td>
<td>8.79$^{a,b}$</td>
</tr>
</tbody>
</table>

$^1$ Steady-state ratio calculated as: (uptake at 10 $\mu M$ $P_i$ in seedlings grown at 10 $\mu M$ $P_i$)/ (uptake at 100 $\mu M$ $P_i$ in seedlings grown at 100 $\mu M$ $P_i$).

$^2$ Affinity ratio calculated as: (uptake at 10 $\mu M$ $P_i$ in seedlings grown at 10 $\mu M$ $P_i$)/ (uptake at 10 $\mu M$ $P_i$ in seedlings grown at 100 $\mu M$ $P_i$).

Ratio is significantly different from 1.00 ($P < 0.01$).

Ratio is significantly different from that of non-inoculated seedlings ($P < 0.01$).
sink strength did not alter P allocation to shoots (Figure 2); however, when grown with 10 μM P, allocation of P between root and shoot was significantly altered as a result of mycorrhizal colonization, reducing P translocation to foliage (Figure 2, Table 1).

Patterns of $^{32}$P uptake by roots of both non-mycorrhizal and mycorrhizal seedlings indicated that there was a high degree of acclimation to P limitation. Rates of $^{32}$P uptake by non-mycorrhizal and mycorrhizal seedlings grown with 10 μM P were greater than rates for seedlings grown with 100 μM P. Furthermore, there were significant interactions involving fungal species and solution $^{32}$P concentrations. Rates of $^{32}$P uptake, when expressed as steady-state and affinity ratios (see Table 2), suggest that, in response to decreasing P availability, non-mycorrhizal roots and roots colonized with L. bicolor, P. involutus, or P. tinctorius all produce more and higher affinity transporters to maintain uptake of P. The steady-state ratios indicate that uptake of $^{32}$P from solutions of low P concentrations is equal to or greater than uptake from solutions of high P concentrations when the seedlings and their associated fungi are acclimated to these conditions. In the case of roots colonized with P. involutus or P. tinctorius, steady-state and affinity ratios suggest that these mycobionts are especially responsive to P limitation, exhibiting greater P uptake rates when seedlings are grown at 10 μM than at 100 μM P (Table 1). Similar patterns have been noted for roots of non-mycorrhizal barley and tomato plants (Clarkson and Scattergood 1982, Lefebvre and Glass 1982) as well as roots of tomato and onion plants colonized with endomycorrhizal fungi (Cress et al. 1979, Son and Smith 1988).

It is important to note that the observed enhancement of $^{32}$P uptake by mycorrhizal roots did not manifest itself as enhanced P translocation to foliage. Colonization by L. bicolor, P. involutus, or P. tinctorius tended to reduce foliar P concentrations when seedlings were grown under P limitation. These findings are in contrast to previous work on pitch pine and P. tinctorius (Cumming and Weinstein 1990, Cumming 1993), where colonization by this mycobiont enhanced seedling P nutrition under P-limiting conditions. Although there is no reason to expect L. bicolor and P. involutus to confer the same beneficial effect to host seedlings, the influence of P. tinctorius on seedling P nutrition and $^{32}$P fluxes in the present study is perplexing. The isolates of P. tinctorius used in the two studies differed, and it may be that intraspecific variation in P allocation under P limitation is a major factor governing potential benefits accrued by host seedlings. Ho (1987) noted 10-, 9-, and 40-fold differences in growth rate, APase activity, and nitrate reductase activity, respectively, among eight isolates of P. tinctorius, reflecting a high degree of physiological variation within this ectomycorrhizal fungus.

Over the long term, the acclimation of seedlings and fungi to P availability may be responsible for the lack of response of root surface APase activity to low P availability (Figure 5). Increases in APase activity are often noted for plant tissue culture systems (Ueki 1978, Duff et al. 1981, Goldstein et al. 1988), in short-term P-deprivation studies (Goldstein et al. 1988), and in intact plant systems where no P is supplied (Caradus and Snaydon 1987). Similarly, changes in APase activity of ectomycorrhizal fungi are often reported for in vitro systems (Ho and Zak 1979, Antibus et al. 1986, Kroehler et al. 1988) where P is absolutely limiting. In long-term experiments, roots colonized with ectomycorrhizal fungi exhibit increased or decreased APase activities in response to P availability, depending on host and fungal species (Dighton 1983, Cumming and Weinstein 1990, Pasqualini et al. 1992, Cumming 1993). The lack of a clear APase response to P deprivation in mycorrhizal roots may be the result of greater P uptake capacities or lower P uptake thresholds, which may suppress an APase response (Bolan 1991).

The present results point to the importance of acclimation to P availability and ectomycorrhizal symbioses in balancing P uptake with demand in pitch pine seedlings. When P was not limiting, there were no differences in P uptake between non-mycorrhizal and mycorrhizal roots, although mycorrhizal roots represented a strong P sink within seedlings. Under P-limiting conditions, P uptake rates increased in all seedlings, and increases were greater in roots colonized with mycorrhizal fungi than in non-mycorrhizal roots. However, the strong sink strength exhibited by the mycorrhizal symbionts reduced allocation of P to seedling shoots. Thus, although more P was absorbed from the environment, it was not used to enhance biomass growth of the host seedling. The changes in P uptake capacity at low and high P concentrations were apparently sufficient to modulate root APase responses to P limitation. Rates of APase were significantly lower in mycorrhizal roots than in non-mycorrhizal roots, indicating that mycorrhizal APase systems do not play a role in overcoming P limitation in pitch pine.

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References


