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Phosphorus nutrition of mycorrhizal trees

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Summary Globally, phosphorus (P) limits productivity of trees in many forests and plantations especially in highly weathered, acidic or calcareous profiles. Most trees form mycorrhizal associations which are prevalent in the organic and mineral soil horizons. This review critically examines mechanisms that enhance the acquisition of P by tree roots. Mycorrhizal roots have a greater capacity to take up phosphate (Pi) from the soil solution than non-mycorrhizal root tips. Factors that contribute to this include the extent of extraradical hyphal penetration of soil and the physiology and biochemistry of the fungal/soil and fungal/plant interfaces. Ectomycorrhizal (ECM) trees are likely to benefit from association with basidiomycetes that possess several high-affinity Pi transporters that are expressed in extraradical hyphae and whose expression is enhanced by P deficiency. To understand fully the role of these putative transporters in the symbiosis, data regarding their localization, Pi transport capacities and regulation are required. Some ECM fungi are able to effect release of Pi from insoluble mineral P through excretion of low-molecular-weight organic anions such as oxalate, but the relative contribution of insoluble P dissolution in situ remains to be quantified. How the production of oxalate is regulated by nitrogen remains a key question to be answered. Lastly, phosphatase release from mycorrhizas is likely to play a significant role in the acquisition of Pi from labile organic forms of P (Po). As labile forms of Po can constitute the major fraction of the total P in some tropical and temperate soils, a greater understanding of the forms of Po available to the phosphatases is warranted.

Keywords: fungal P transporters, gene expression, organic and mineral phosphorus mobilization, organic anions, phosphatase release, phosphate uptake.

Introduction

This review explores how mycorrhizal associations enhance the uptake of phosphorus (P) by trees. However, before doing so, some introductory remarks are necessary to place in context the mycorrhizal studies on functional interactions between specific tree–fungal associations concerning P acquisition.

First, P limits the productivity of plants in many terrestrial ecosystems (Batjes 1997) and is often the first or second element limiting aboveground net primary productivity (ANPP) of forests. The supply of P to tree roots is particularly constrained in acid soils, calcareous/alkaline soils and old, highly weathered soils. A world map of P-limiting soils has been published recently (Lynch and Brown 2008). Acid soils occupy about 30% of the world's land surface, and over half of this area supports forests and woodlands in the northern cold and temperate zone (Alfisols, Histosols, Inceptisols, Spodosols) and in the tropics (Oxisols, Ultisols) (von Uexküll and Mutert 1995). Phosphorus deficiency in the latter soils (Walker and Syers 1976) is usually attributed to the occlusion of inorganic P by oxides of Al and Fe (Herbert and Fownes 1995). Meta-analysis of experimental enrichment studies has shown that forests in the tropics have a stronger response to added P than nitrogen (N) (Elser et al. 2007). Field studies are beginning to confirm the extent of P limitation in lowland tropical forests. For example, correlations between soil P and tree growth in natural forests in Kalimantan support the view that P is the primary driver for spatial patterns of ANPP in the landscape (Paoli and Curran 2007). Studies of long-term chronosequences indicate that P is more limiting for systems that have been free of disturbance, such as glaciers or volcanism, for long periods (Richardson et al. 2004, Wardle et al. 2004). Conversely, disturbance of forests can shift limitations away from P towards N due to the greater mobility of N in soil (Herbert et al. 2003). It has been suggested that in areas of gradual uplift, not subject to intense erosion, advection of P into the root zone may contribute to P uptake by plants (Porder et al. 2007).

Secondly, the importance of organic forms of P in soils to tree nutrition is likely to have been underestimated due to the limited number of studies relative to those documenting inorganic P behaviour, chemistry, capture and uptake. The slow diffusion of orthophosphate ions (Pi = HPO_4^{2-}, $H_2PO_4^{1-}$) in soil solution, the large concentration gradient between the bulk soil and root surface and development of depletion zones around roots as a result of Pi uptake are well known

(Bieleski 1973, Tinker and Nye 2000, Hinsinger 2001). By contrast, there is a wide range of organic P (Po) compounds that differ in biological availability to roots (Condron et al. 2005) that have yet to be fully understood in forest soils. These include mono- and di-phosphate esters such as nucleic acids and phospholipids (Leake and Miles 1996, Myers and Leake 1996), organic polyphosphates and inositol phosphates (Turner 2008), amongst others. Labile forms of Po can constitute the major fraction of the total P in some tropical and temperate soils. Typically, Po contributes from 30 to 65%, sometimes >90%, of the total soil P (Harrison 1987). The turnover of Po in soil is primarily determined by immobilization and mineralization rates (Condron and Tiessen 2005). Pritchett and Fisher (1987) suggest that annual recycling of P in plant residues above and belowground can contribute 15–80% of the P uptake in forest ecosystems. In a recent study in Panama, Vincent et al. (2010) have shown that Po in mineral soil makes an important contribution to the nutrition of a semi-evergreen tropical forest. However, the relative availability of inorganic and organic P forms depends on many factors including soil reactivity, depth and moisture. For example, Achat et al. (2009) assessed the potential contribution of organic and inorganic P to plant-available P in a *Pinus pinaster* Aiton plantation on highly P-deficient soil and concluded that the contribution from the organic P fraction was predominant in the litter and negligible below 30 cm.

Thirdly, given the Pi supply constraints in many soils, it is not surprising that plants have evolved strategies to acquire and/or efficiently use P. Plant adaptations that enhance the acquisition of inorganic Pi have been reviewed many times (e.g., Raghothama 1999, Vance et al. 2003, Bucher 2007, Lambers et al. 2008, Richardson et al. 2009). Such adaptations include modifications to root structure and morphology as well as biochemical (e.g., root exudates) and molecular mechanisms. Approximately 80% of land plants form mycorrhizal associations in their natural habitats and thus are likely to acquire P via fungal networks in the soil and litter. Although the vast majority of tree species are mycorrhizal, the type of association differs greatly between geographical regions, being predominantly ectomycorrhizal in forests in tropical and subtropical Asia, for example, but endomycorrhizal in tropical lowland forests in South America. Furthermore, due to the relatively extensive, near-surface, fine root systems of trees, opportunities abound for the coexistence of a great diversity of mycorrhizal associations in space and in time. We understand little about the relative contribution of these mycorrhizal mosaics to the inorganic and organic P nutrition of trees. Furthermore, opportunities may exist for co-existing plant species to partition soil organic P to reduce competition (Turner 2008).

Uptake of phosphate by fungal hyphae

To overcome the Pi limitation in the rhizosphere, the formation of symbiotic structures with mycorrhizal fungi is considered as the most widespread response to increase P acquisition by plants (Smith et al. 2000, Burleigh et al. 2002, Tibbett and Sanders 2002), as illustrated by the repeated observation that mycorrhizal plants accumulate more P than non-mycorrhizal plants (Smith and Read 1997, 2008, Chalot et al. 2002). As mentioned above, with the exception of the Dipterocarpaceae, many trees from the tropics are associated with arbuscular mycorrhizal (AM) fungi, whereas trees from the gymnosperms and several angiosperm groups growing in boreal and temperate regions form ectomycorrhizal (ECM) roots. Whatever the mycorrhizal type (AM or ECM), mycorrhizal fungi produce extraradical hyphae that are able to explore the soil away from the root and provide a conduit to the root where exchanges between fungal and root cells occur. Extraradical hyphae are thought to play a major role in overcoming the Pi depletion zone as they are able to increase considerably the volume of soil exploited by mycorrhizal plants. As an example, data obtained in young pot-grown *Pinus taeda* L. showed that the absorbing surface contributed by the hyphae of two ECM fungal species represented about 75% of the uptake potential of the root system (Rousseau et al. 1994).

The first demonstration of Pi uptake by extra-matrical hyphae and its subsequent transfer towards the host plant was carried out using ^{32}P-Pi supplied to young *Pinus sylvestris* L. plants grown under sterile conditions (Melin and Nilsson 1950). The pattern of ^{32}P accumulation measured in excised roots of *Pinus radiata* D. Don, incubated for 15 min in a solution containing 5 μM KH_2PO_4, varied greatly according to the mycorrhizal status of the root (Bowen 1968 in Mousain et al. 1997). In non-mycorrhizal roots, ^{32}P accumulation occurred mainly in the growing root zone (0.5–4 cm from the root tip) and decreased strongly when the root became suberized. The presence of non-mycorrhizal short roots only slightly increased ^{32}P accumulation. In contrast, when the root was bearing ECM tips, ^{32}P accumulation was dramatically increased in these tips (Bowen 1968 in Mousain et al. 1997). These data demonstrated the high capacity of ECM tips to take up Pi from the solution compared with non-mycorrhizal root tips.

In addition to the uptake capacities displayed by ECM roots, studies carried out with intact plants grown in microcosms showed that extraradical hyphae and mycelial strands of *Suillus bovinus* (Pers.) Roussel interconnecting *Pinus contorta* Douglas and *P. sylvestris* plants were also able to take up ^{32}P-Pi and to translocate labelled P to the shoots (Finlay and Read 1986). This transport of P is unidirectional (from the fungal to the host cells) as no translocation of ^{32}P supplied to non-mycorrhizal roots was able to be detected in *S. bovinus* mycelium (Finlay and Read 1986). In another study, P uptake and translocation through intact mycelial systems of *Paxillus involutus* (Batsch) Fr. and *Suillus variegatus* (Sw.) Kuntze associated with *P. contorta* grown in microcosms was measured non-destructively using a β-scanner (Timonen et al. 1996). The pattern of ^{32}P-phosphate accumulation as a function of time showed that the fine, foraging hyphae were more efficient to take up Pi than the cut mycelial strands.

Table 1. Kinetic parameters of the high-affinity Pi uptake system in intact mycorrhizal and non-mycorrhizal *P. sylvestris* root systems. Adapted from Van Tichelen and Colpaert (2000).

Plant age (weeks)	Inoculation treatment	Apparent K_m (μM Pi)	Apparent V_{max} (nmol g^{-1} s^{-1})
7	*Paxillus involutus*	3.5 (2.4–4.5)[1]	0.57 (0.50–0.66)
	Suillus bovinus	7.5 (5.1–10.1)	0.49 (0.45–0.55)
	Thelephora terrestris	8.7 (6.5–10.1)	0.13 (0.09–0.17)
9	*Paxillus involutus*	5.9 (4.1–7.2)	0.62 (0.48–0.70)
	Suillus bovinus	10.2 (7.2–12.3)	0.52 (0.48–0.54)
	Thelephora terrestris	7.3 (4.6–9.9)	0.15 (0.10–0.19)
	None (non-mycorrhizal)	12.1 (7.8–16.4)	0.08 (0.07–0.10)

[1]Means and range of variation (between brackets) are shown.

Studies with plants that are able to host dual associations suggest that ECM fungi are more efficient than AM in acquiring and transferring P to roots (Jones et al. 1998, van der Heijden 2001). This may in part be due to the architecture of the hyphal network as the largest growth promotion and increase in seedling P content following ECM association of *Eucalyptus coccifera* Hook. with *Laccaria bicolor* (Mre) Ort. or *Thelephora terrestris* Ehrh. was highly correlated with the development of external hyphae, which was three to seven times higher than that of the three *Glomus* species used (Jones et al. 1998). However, the effect of *Glomus mossae* (Nicol. & Gerd.) Gerd. & Trappe on growth and P uptake of *Salix repens* L. cuttings depended on the P status of the leaves. The beneficial effect of AM colonization, although occurring at a low rate of colonization (<5%), was greater and faster when the leaves had a low P status than when they had a high P status (van der Heijden 2001). These results suggest that mycelial P uptake and transfer from AM hyphae could depend on the P status of the host plant.

For example, excess or very low soil N has been shown to reduce the amount of external hyphae estimated from ergosterol concentrations assayed in an artificial substrate, whereas Pi starvation had the opposite effect (Wallander and Nylund 1992). However, increasing Pi availability in controlled conditions resulted either in positive (Brandes et al. 1998) or negative (Jones et al 1998, Torres Aquino and Plassard 2004) effects on mycelial growth. The same trends were obtained in the field, with an increase (Bakker et al. 2009) or a decrease (Pampolina et al. 2002) in hyphal length measured after the application of P fertilizer in maritime pine or eucalypt plantations, respectively. These opposite results could be related to the actual bioavailability of Pi, which may be highly different from the rate of applied fertilizer. The distribution of fertilizer P may also be a factor as experiments by Brandes et al. 1998 have shown localized increases in hyphal density in response to nutrients in situations where the plant roots have not been exposed directly to increased levels of nutrients.

The role of external hyphae associated with trees was further quantified by measuring kinetic parameters of Pi uptake rates in young *P. sylvestris* grown in perlite. The Pi uptake capacity of the pine root system was strongly enhanced by the ECM association by decreasing the K_m and increasing V_{max} of Pi uptake rates compared with non-mycorrhizal plants (Table 1, Van Tichelen and Colpaert 2000). On the other hand, results obtained with *Hebeloma cylindrosporum* (Bull.) Quél./*P. pinaster* plants grown in soil conditions showed that accumulation of P in whole ECM plants significantly correlated with soil exploration by external hyphae, suggesting that plant P mainly originated from fungal P uptake (Torres Aquino and Plassard 2004) as demonstrated in AM plants (Smith et al. 2003, 2004). Under this assumption, the net P transfer from *H. cylindrosporum* to the plant was estimated as 0.36 and 0.66 μmol of P per cm^2 of mycelium in soil of low and high P availability, respectively.

Fungal phosphate transporters

So far, in fungi, two types of high-affinity Pi transporter have been characterized that are either $Pi:H^+$ or $Pi:Na^+$ transporters. In yeast, these two transporters are encoded by two genes, *PHO84* (Bun-ya et al. 1991) and *PHO89* (Martinez and Persson 1998), whose expression is activated when the cells meet a limitation in external Pi (Persson et al. 2003). The PHO84 transport system displayed K_m values for external Pi ranging from 1 to 15 μM, whereas PHO89 displayed a K_m for external Pi of 0.5 μM (Persson et al. 2003). In mycorrhizal fungi, the first data regarding the identification of phosphate transporters possibly involved in Pi uptake were obtained in the AM species *Glomus versiforme* (Karsten) Berch associated with *Medicago truncatula* Gaertn. (Harrison and van Buuren 1995). When expressed in a yeast mutant lacking PHO84, this transporter (named GvPT) displayed a Pi transport activity prevented by carbonyl-cyanide-*m*-chlorophenyl-hydrazone (CCCP, which uncouples the proton gradient across the membrane), suggesting that the transporter is operating via a proton-coupled symport. Phosphate uptake mediated by GvPT in yeast exhibited an apparent K_m value of 18 μM Pi (Harrison and van Buuren 1995). Further, one partial cDNA (*GmosPT*) and one full-length cDNA (*GiPT*) putatively coding for Pi transporters have been identified in two AM species, *G. mossae* and *Glomus intraradices* Schenck & Smith, respectively (Maldonado-Mendoza et al. 2001, Benedetto et al. 2005). All these

transcripts have been predominantly detected in extraradical hyphae, with their expression level enhanced by low P availability, such as reported in *G. intraradices* (Maldonado-Mendoza et al. 2001, Olsson et al. 2006) and *G. mossae* (Benedetto et al. 2005). Taken as a whole, these data suggest a role in Pi acquisition from the soil. However, it should be noted that Benedetto et al. (2005) and Balestrini et al. (2007) have reported that transcripts of *GmosPT* are detected also in intraradical hyphae and in cells containing arbuscules, suggesting that the regulation of P uptake and transfer from the fungal to the host cells is far more complex than previously expected.

Regarding ECM fungi, five genes possibly coding for Pi transporters have been identified in the genome of *L. bicolor*, recently completely sequenced (Martin et al. 2008), and two other genes, *HcPT1* and *HcPT2*, have been identified in another ECM species, *H. cylindrosporum* (Tatry et al. 2009). Remarkably, all the genes identified in mycorrhizal fungal species clustered with the fungal Pi:H^+ transporters (Figure 1), suggesting that the efficiency of Pi uptake into mycorrhizal fungal cells could rely strongly upon the external pH. The five genes identified in *L. bicolor* have not been characterized yet. However, this has been done for *HcPT1* and *HcPT2*, which are close to *L. bicolor* genes (Figure 1). The complete cDNAs, identified from a cDNA library (Lambilliotte et al. 2004), were expressed in a yeast mutant lacking PHO84 and functionally characterized (Tatry et al. 2009).

Measurements of ^{33}P-Pi influx showed that the two encoded polypeptides were able to mediate Pi:H^+ symport with different affinities for Pi, the K_m values being 55 and 4 μM, respectively, for HcPT1 and HcPT2. The apparent K_m of HcPT2 was therefore comparable with that reported for PHO84 and lower than that of the gene *GvPT* (18 μM), which is the only value for a Pi transporter of mycorrhizal fungi currently available for comparison (Harrison and van Buuren 1995). Conversely, the value of K_m found for HcPT2 was in the same range as those found in intact plants of *P. sylvestris* (see Table 1), suggesting that this phosphate transporter could play a great role in Pi uptake into fungal cells. Expression levels of *HcPT1* and *HcPT2* were quantified as a function of external Pi availability in the hyphae grown in pure culture or associated with their host plant *P. pinaster*. Levels of *HcPT1* transcripts were always higher in fungal cells exposed to Pi starvation in solution or to low Pi availability in soil, suggesting that the regulation of this transport system is close to that of PHO84 in yeast. In contrast, transcript levels of *HcPT2* were less dependent on Pi availability in fungal cells grown in vitro and were upregulated in ECM roots grown in soil with high P availability (Tatry et al. 2009).

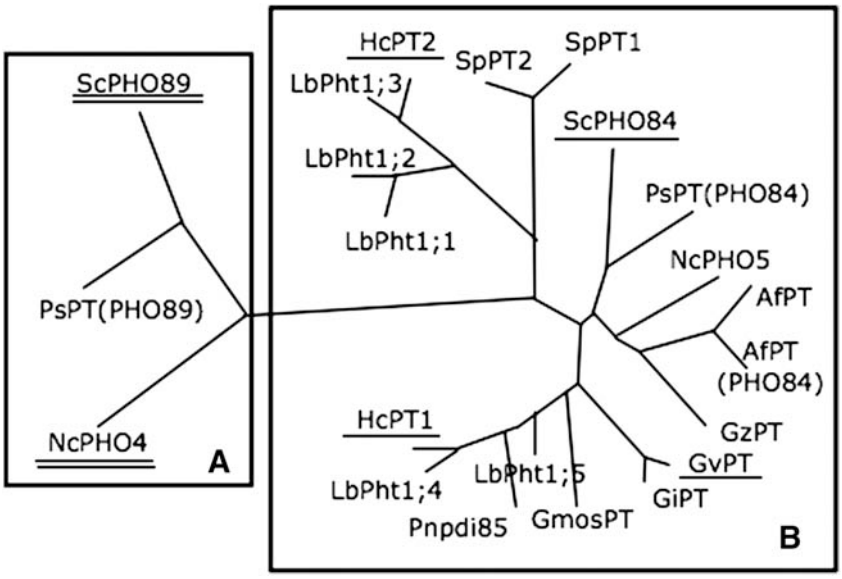

Figure 1. Mycorrhizal fungi predicted polypeptides cluster with fungal Pi:H+ symporters. The unrooted phylogenetic tree was constructed from predicted protein sequences for fungal P transporters of the following species: *Aspergillus fumigatus* Fres. (AfPT XM741455), *Aspergillus terreus* Thom (AtPT(PHO84) XM001217523), *Gibberella zeae* (Schwein.) Petch (GzPT XP_388070), *G. intraradices* (GiPT AF359112), *G. versiforme* (GvPT U38650), *H. cylindrosporum* (HcPT1 AJ970310, HcPT2 AJ970311), *Laccaria bicolor* (LbPht1,1-5 retrieved from JGI, http://genome.jgi-psf.org/Lacbi1/Lacbi1.home.html), *Neurospora crassa* Shear & B.O. Dodge (NcPHO4 M31364, NcPHO5, L36127), *Pichia stipitis* Pignal (PsPT(PHO84) XM001383316, PsPT(PHO89) XM001387528), *Pholiota nameko* (T. Ito) S. Ito & S. Imai (Pnpdi85 AB060641), *Saccharomyces cerevisiae* Meyen (ScPHO84 BAA14358, ScPHO89 NP_009855), *Schizosaccharomyces pombe* Lindner (SpPT1 O42885, SpPT2 NP_595057). Double- and single-underlined polypeptides have been functionally characterized as Pi:Na + and Pi:H+ symporters, respectively (Bun-ya et al. 1991, Harrison and van Buuren, 1995, Versaw and Metzenberg, 1995, Martinez and Persson, 1998). According to these characterized phosphate transporters, polypeptides likely to have Pi:Na+ symport activities cluster in group (A), those with Pi:H+ symport activities in group (B). Note that all the predicted polypeptides identified so far in mycorrhizal fungi, whether AM (GvPT, GiPT and GmosPT) or ECM (HcPT1-2, LbPht1;1-5) cluster in group (B).

Taken as a whole, these results indicate that *H. cylindrosporum* might use HcPT1 to mediate Pi uptake when soil P availability is low and HcPT2 when soil P availability is high. However, one has to explain the discrepancy between the rather high apparent affinity value measured for HcPT1 expressed in yeast and its functioning when Pi availability is low. As reported by Tatry et al. (2009), one hypothesis could be that heterologous expression of *HcPT1* in yeast may result in an overestimation of the apparent K_m value. This explanation was suggested for StPT1, a potato Pi transporter with an apparent K_m of 130 µM determined in yeast and an expression in plant roots that was upregulated by Pi starvation (Leggewie et al. 1997). The authors proposed that the proper function of high-affinity Pi transporters may require additional subunits that are absent when expressing a putative Pi transporter in a heterologous system, for example yeast. In agreement with this hypothesis is the observation that the apparent K_m of ScPHO84 increases from 8 to 24 µM when the transporter is operating in native conditions (yeast; Bun-ya et al. 1991) or in artificial conditions (proteoliposomes; Berhe et al. 1995), respectively.

Release of organic anions

In addition to their capacity to take up inorganic phosphate from the soil solution, ECM fungi, as many other fungal species, may release organic acids that are low-molecular-weight CHO-containing compounds (LMWOAs) characterized by the possession of one or more carboxyl groups (Dutton and Evans 1996, Jones 1998). The low-molecular-weight organic acids, bearing one or several negative charges, are able to release Pi from insoluble mineral P through complexolysis (Courty et al. 2010) where negatively charged molecules attach to mineral cations through electrostatic and covalent forces (Haas and Purvis 2006). This reaction leads to the solubilization of nutrients from the mineral surface such as apatite. In addition, the LMWOAs can be released into the external medium with protons (Arvieu et al. 2003). These protons result in acidolysis of minerals by dismantling the crystalline structure of the mineral (Courty et al. 2010). Therefore, LMWOAs are considered as the main agents of mineral dissolution because of their complexing and acidifying properties (Barker et al. 1998).

To date, most of the results regarding LMWOA production have been obtained in ECM fungi as only two studies are available on the production of organic acids in AM fungi (Plassard and Fransson 2009), showing that acetate and formiate (Toljander et al. 2007) and citrate and malate (Tawaraya et al. 2006) have been detected in hyphal exudates of the latter fungi. Among the approximately 30 species of ECM fungi that have been studied so far, species belonging to the genera *Cortinarius*, *Lactarius*, *Paxillus*, *Piloderma*, *Pisolithus* and *Suillus* were found to be able to release substantial amounts of LMWOAs, with oxalate being the predominant form (Courty et al. 2010). Nevertheless, a huge intraspecific variation in the capacity to release organic acids exists among these LMWOA-producing fungal genera, as shown for *P. involutus* by Lapeyrie et al. (1991). On the other hand, almost no production of organic acids was detected in some ECM species belonging to the genera *Amanita*, *Cenococcum*, *Hebeloma*, *Thelephora* and *Tylospora* (Courty et al. 2010).

The positive role of ECM symbiosis on the use of mineral P by the host plant has been studied by adding apatite (e.g., Wallander et al. 1997, Wallander 2000, Casarin et al. 2004, Rosling et al. 2004, Smits et al. 2008) or rock phosphate (e.g., Liu et al. 2008). Using *P. sylvestris* seedlings mycorrhizal with *P. involutus* in agar-based microcosms, Smits et al. (2008) showed that ECM fungi preferentially allocate carbon to hyphae with access to minerals containing P. This strategy may avoid a waste of energy by increasing the selective colonization of P-containing patches in mineral soil. ECM fungi can also selectively colonize minerals lacking P (Rosling et al. 2004).

The addition of mineral P always increased the amount of P accumulated in mycorrhizal plants compared with non-mycorrhizal ones. However, different efficiencies to improve host P nutrition were observed among inoculation treatments that can be attributed to different abilities of each fungal species to release oxalate. Indeed, the study carried out by Casarin et al. (2003) demonstrated that the ability to release oxalate by a fungal species grown in vitro was increased when the fungus was associated with a host plant. In this study, no oxalate was detected in rhizosphere soil of *P. pinaster* plants, whether non-mycorrhizal or associated with *H. cylindrosporum*, a very poor producer of oxalate (Arvieu et al. 2003). In contrast, oxalate was always measured in the rhizosphere of plants associated with *Rhizopogon roseolus* (Corda) Th. Fr. (Casarin et al. 2003), a very good oxalate producer in pure culture (Arvieu et al. 2003). Easily extractable Pi concentrations (Olsen P) measured in the same soil samples were positively correlated with oxalate concentrations and negatively correlated with pH and were always higher when apatite was added to the soil (Figure 2, Casarin et al. 2004). A positive relationship between Olsen P and oxalate concentrations in soil samples was also observed with *P. sylvestris* associated with two isolates of *S. variegatus* and one unidentified ECM fungal species and grown with apatite accessible only to the hyphae (Wallander 2000). Overall, the results reported from these studies carried out in controlled conditions with simplified (Wallander 2000) or sterilized (Casarin et al. 2003, 2004) soil strongly support a central role played by oxalate in the release of Pi from apatite.

Several environmental factors may increase or decrease organic acid production by ECM fungi. Nitrogen source appears to be a major factor as in vitro studies conducted in pure culture show a consistent stimulation of oxalic acid production in the presence of nitrate and inhibition by ammonium for the ECM species *P. involutus* (Lapeyrie et al. 1987, 1991, Gharieb and Gadd 1999). We can hypothesize that it is the NH_4^+ ion per se that has an inhibitory effect on oxalate synthesis as organic nitrogen did not inhibit organic

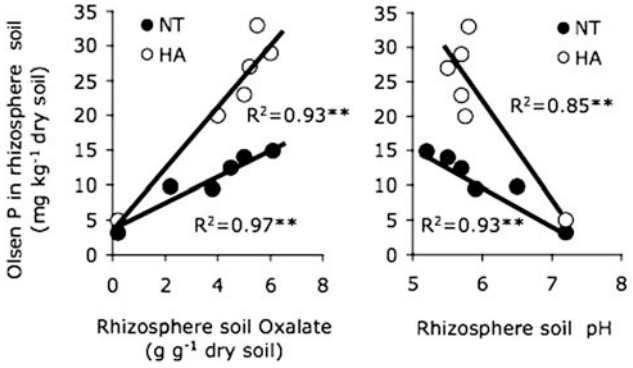

Figure 2. Relationship between easily available P (Olsen P) content and oxalate content (left panel) or pH in rhizosphere soil (right panel) after culture of *P. pinaster* plants associated with *R. roseolus* for 3 months in rhizoboxes containing a thin layer of a Mediterranean chromic cambisol used either intact (NT) or amended with 350 mg P kg^{-1} dry soil supplied as hydroxyapatite (HA). Double asterisks indicate a highly significant regression (at $P < 0.01$) (adapted from Casarin et al. 2004).

acid release in *R. roseolus*, contrary to ammonium (Plassard, unpublished data). When nitrate is supplied as the sole nitrogen source in the medium, the presence of calcium and bicarbonate ions was shown to promote oxalate production in the fungus grown in pure culture (Lapeyrie et al. 1987) or in association with the plant (Casarin et al. 2003, 2004). As shown by Lapeyrie (1988), the carbon from bicarbonate is incorporated into the oxalate molecule synthesized by *P. involutus*, and available bicarbonate could therefore act as a limiting substrate especially in acidic situations. In contrast, oxalate could be produced in large amounts in calcareous situations characterized by high nitrification rates and high levels of bicarbonate. This oxalate release, coupled with proton release, could therefore be of great importance for the release of Pi from insoluble mineral P forms that are combined with calcium.

However, the estimation of the relative contribution of ECM fungi in insoluble P dissolution in situ remains a challenging question. Indeed, a positive relationship between oxalate concentration and available P is not always observed in ECM plants, despite the improvement of plant P status by ECM symbiosis (see e.g., Liu et al. 2008). Concentrations of oxalate reported so far are highly variable, and this could be due at least to the specific variability of ECM species in releasing oxalate and/or the available nitrogen source (see above), combined with a high turnover rate due to microbial use of organic anions (Jones 1998, van Hees et al. 2003). Nevertheless, in natural conditions, oxalate release by ECM fungi should play an important role in mineral P dissolution. Progress in this field is hampered by the lack of an easy method to measure LMWOAs in field-sampled ECM fungi. Recently, Rineau et al. (2008) reported a microplate assay making it possible to measure the release of oxalate at the level of individual ECM tips. The use of this method could be a very valuable tool to assess at least the capacities of oxalate released by ECM species even if they are not grown in vitro. The comparison of oxalate release capacity by these ECM tips with the actual concentration of oxalate in soil sample surrounding these tips could help to quantify the fate of LMWOAs released into the mycorhizosphere.

Release of P from organic P compounds

Remarkably, besides the low level of available free Pi, soils contain a high amount of P that is linked to C-containing compounds to form Po. The majority of the organic phosphorus is present as phosphate esters (C–O–P bonds) either in the form of phosphate monoesters, including inositol phosphates, or phosphate diesters, such as nucleic acids and phospholipids, together with small quantities of phosphonates (C–P bonds) (Magid et al. 1996, Condron et al. 2005). To be used by plants and soil microorganisms, the phosphate group of Po compounds must be released from the ester bond linking it to carbon by enzymes that are phosphatases. Depending on their substrate, the enzymes can be phospho-monoesterases or phospho-diesterases. Most of the studies addressing the release of phosphatases use artificial phospho-monoesters such as *p*-nitrophenyl phosphate (pNPP) based on the procedure described in Tabatabai and Bremner (1969) or the fluorescent assay based on the release of 4-methylumbelliferone from 4-methylumbelliferone-phosphate (Courty et al. 2005) to estimate phosphomonoesterase activity. Depending on the pH of the incubation medium, one can distinguish acid phosphomonoesterase activity (ACP) from alkaline phosphomonoesterase activity (ALP), measured respectively at pH around 5 (ACP) and >8 (ALP). The release of ACP activities into the environment has been shown in many microorganisms, including various soil fungi (Nahas et al. 1982, Bae and Barton 1989, Haas et al. 1992). Regarding mycorrhizal fungi, active release of phosphatases into the soil has been questioned for arbuscular fungi (Joner et al. 2000). However, the stimulation of plant ACP release by roots of *Tagetes patula* L. associated with *Glomus etunicatum* Becker & Gerdemann has been reported by Ezawa et al. (2005). These results led the

Table 2. Fate of P contained in litter, pollen or nematode necromass added to microcosms containing one *Betula pendula* Roth. seedling per microcosm, whether associated (M) or not (NM) with the ECM fungus *Paxillus involutus*. Data from Perez-Moreno and Read 2000 (1), 2001a (2) and 2001b (3).

Treatment	Experiment duration (days)	Amount of P (μg)			Estimated Po lost (%)[1]	Ref
		Added as input	Lost during experiment	Accumulated in plant		
Birch litter	90	388	155		65	1
Pine litter		293	104		68	
Beech litter		301	111		71	
Total[2]		982	369	125	69	
Pollen, NM	115	1367	472	93	na[3]	2
Pollen, M		1367	1324	344	na	
Nematodes, NM	195	223	55	48	na	3
Nematodes, M		223	147	107	na	

[1]Amount of P reduction attributable to loss from the organic fraction (Po), based upon the difference between extractable and total P contained in litter at the beginning and the end of the experiment.
[2]Each microcosm contained the three litter types for one single mycorrhizal plant.
[3]Not applicable.

authors to propose a new hypothesis to improve P acquisition by AM plants, which is the up-regulation of the secreted acid phosphatase gene from the host plant (Ezawa et al. 2005).

Such an effect of ECM fungi on the up-regulation of expression of ACP from the host plant has not been studied yet. However, in contrast to AM plants, ECM fungi have been shown to release ACP in pure culture (e.g., Tibbett et al. 1998, Louche et al. 2010). As a result, ECM plants often increase the phosphatase activity in the rhizosphere soil or around ECM tips (Buée et al. 2005, Courty et al. 2006), and this is occasionally related to the degradation of labile organic P (see e.g., Liu et al. 2004). In experiments carried out in microcosms, Bending and Read (1995) measured higher levels of ACP in fermentation horizon organic matter (FHOM) colonized by young mycelium of *P. involutus* than in those not colonized or colonized by old mycelium, indicating the ability of this fungus to release ACP into its environment. The scavenging efficiency of *P. involutus* to mobilize P from complex materials was confirmed later on. As shown in Table 2, this fungal species was able to strongly deplete the P content of litter from birch, pine or beech, pollen or nematode necromass supplied in small trays placed in microcosms. Whatever the external source added to the microcosms, *P. involutus* intensively exploited this source of nutriments by colonizing selectively these nutrient-rich patches (Perez-Moreno and Read 2000, 2001*a*, 2001*b*). This efficiency in mobilizing P was not dependent upon the plant species input, as the same loss of P (around 30% in 90 days) was measured from birch, pine and beech litter. Of this lost amount of P, 33% was transferred to the host plant. The remainder may have been accumulated in the biomass of *P. involutus* for sustaining soil exploration and enzyme production or could have been used by other microorganisms. Finally, the authors calculated the P lost from organic P contained in litter as close to 70%. Using the same experimental system, Perez-Moreno and Read (2001*a*) showed that *P. involutus* intensively exploited a pollen source, resulting in a reduction of 97% of the initial P content (Table 2). Of this, 25% of the P was transferred to the host plant. In non-mycorrhizal microcosms, only 35% of P was lost from pollen, presumably as a result of export by fungal saprotrophs, and only 7% of this P was recovered in the plants (Perez-Moreno and Read 2001*a*). Taken as a whole, these results indicate that the fungus is able to mobilize P from pollen (Read and Perez-Moreno 2003). Remarkably, *P. involutus* was even more efficient in transferring P mobilized from the necromass of nematodes, the P amounts measured in mycorrhizal host plant reaching up to 73% of the total P lost from nematodes (Table 2). However, it should be noticed that non-mycorrhizal plants were also able to take up 87% of the total P lost from nematodes (Table 2). Whether this reflects the composition of P compounds contained in nematodes (e.g., Pi, simple phosphorylated sugars) remains to be determined. Regarding interactions of ECM fungi and other organisms, experiments confronting ECM species (*P. involutus* or *S. variegatus*) with a wood decomposer (*Hypholoma fasciculare* (Huds.) P. Kumm.) in microcosms showed that the two ECM species were able to capture a significant net amount of ^{32}P from P supplied as ^{32}P-Pi to the saprotrophic one (Lindahl et al. 1999). In contrast, a very low net transfer of ^{32}P supplied to the ECM mycelia occurred in the saprotrophic species (Lindahl et al. 1999). However, the efficiency of the net capture of P by the ECM species depends on weak development of the saprotrophic species (Lindahl et al. 2001). Although the physiological mechanisms sustaining this net capture of P are yet to be elucidated, this additional source of P may be important for ECM trees under field conditions.

ACP has been shown to be regulated more strongly by Pi availability (Pi starvation) than by Po availability. Interestingly, N fertilization of forest plots was shown to increase ACP measured at the level of individual root tips (Taniguchi et al. 2008). In a recent study, van Aarle and

Plassard (2010) studied the variation of ACP in the rhizosphere soil, in ECM tips and in the extraradical hyphae of *H. cylindrosporum* associated with *P. pinaster* and ALP only in extraradical hyphae. Plants were grown in rhizoboxes containing a thin layer of soil with low or high Pi availability. The results showed that acid phosphatase activity of the soil and the root increased with mycorrhizal association, although ACP assayed in all fractions decreased over culture time. In contrast, the proportion of hyphae exhibiting acid phosphatase activity in the extra-matrical mycelium increased over time, whatever the soil Pi level. The same trend was observed for alkaline phosphatase activity, but the gradually increasing proportion of hyphae in the extra-matrical mycelium exhibiting alkaline phosphatase activity, particularly under low P conditions, indicates an induction of alkaline phosphatase activity by P limitation. These results show that the roots and the extraradical hyphae are the main production areas for acid phosphatase activities (van Aarle and Plassard 2010).

Surprisingly, high phosphatase activities measured at the level of ECM roots may not be sufficient to mobilize Pi from Po fractions contained in intact soil. Experiments carried out by growing young maritime plants in an intact podzol, characterized by a very low level of available Pi and a greater pool of Po easily extractable by $NaHCO_3$, showed that ECM association was not able to improve the P nutrition of the host plant (Ali et al. 2009). These results could be due to a low efficiency of the enzymes in breaking down the Po compounds present in the podzol. Indeed, it has been suggested they could be involved in recapturing of excreted plant or fungal compounds (Barrett-Lennard et al. 1993). These phosphatases could also possibly break down and recycle the phospholipids from old hyphae (Nygren and Rosling 2009). Alternatively, if some Pi has been liberated upon the action of fungal phosphatase, strong competition between the ECM plant and other microbial populations, or soil adsorption, may have occurred. Compared with the positive results obtained with ECM fungi such as *P. involutus*, the results from Ali et al. (2009) may indicate that the enzymes released by ECM fungi are able to play a significant role mainly on Po compounds contained in fresh organic matter (such as fermentation horizon organic matter, pollen or microfauna necromass) rather than on Po compounds associated with mineral soil particles.

Concluding remarks

From this review, it is clear that a large amount of knowledge has been obtained regarding the mechanisms that enable mycorrhizal trees to improve their acquisition of P from different P sources and under different conditions. The first mechanism is the increase of Pi uptake through the extent of extra-matrical hyphae that penetrate past the P depletion zone surrounding the roots and access new areas containing available Pi. The uptake of this supplementary Pi from soil solution occurs through fungal Pi transporter(s) localized at the fungal/soil interface. It seems that ECM basidiomycetes possess several high-affinity Pi transporters that may play a role in Pi uptake from soil solution, in contrast to AM fungi (*Glomus* sp.) in which only one high-affinity Pi transporter has been identified so far. In addition, this fungal Pi transporter has been detected inside the roots, in cells containing arbuscules. Collectively, these data underline that we are still far from fully understanding the molecular mechanisms involved in the uptake by and release of P from the fungal cell towards the host cell. Data coming from the sequencing of the complete genome of mycorrhizal fungi, such as *G. intratradices* or *H. cylindrosporum* (see http://www.jgi.doe.gov/genome-projects/pages/projects.jsf), will be of great value to establish how many fungal Pi transporters are possibly involved in the symbiosis. However, to understand fully the role of these putative transporters in the symbiosis, it will be necessary to obtain data regarding their Pi transport capacities and how they are regulated, as well as data on their localization. Ideally, the use of fungal strains deficient in a given Pi transporter should help to assess their actual contribution to P acquisition by the plant. This challenging possibility could be achieved using the RNA silencing technique (RNAi) in mycorrhizal fungi based on the methodology recently successful in *L. bicolor* (Kemppainen et al. 2008). However, this will require the extension of these molecular tools such as RNAi to other mycorrhizal fungal species. Regarding the mobilization of insoluble mineral P by mycorrhizal fungi, the capacity to produce and to release LMWOAs such as oxalate appears of great importance to enhance P availability. However, how the production of oxalate is regulated by nitrogen source remains a key question to be answered. Measurement of oxalate production capacities by ECM tips (Rineau et al. 2008) under various environmental conditions, together with the determination of available sources of N (that could be identified by laboratory incubation studies) should help us to improve our knowledge of this regulation in the field. Finally, regarding the mobilization of organic P, the main question is to know which forms of organic P are really available to the phosphatases released by ECM fungi. To answer this question, special attention should be paid to the actual Pi release from Po compounds in organic matter, microbial biomass or microfaunal necromass, compared with those complexed with mineral particles. This efficiency could be determined by combining nuclear magnetic resonance studies enabling us to identify Po compounds and enzymatic studies carried out with purified enzymes obtained from ECM fungi.

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