

Phosphorus and base cation nutrition, heavy metal accumulation and water relations of ectomycorrhizal plants

Introduction

The early view, effectively expounded by Frank (1894), that ectomycorrhizas were especially important in absorption of nitrogen (N) held sway for much of the early part of the twentieth century. Its prominence was weakened by the seminal paper of Hatch (1937), who demonstrated that mycorrhizal colonization in *Pinus strobus* led to increased concentrations of phosphorus (P) and potassium (K), as well as of N (Table 10.1). Hatch was of the opinion that the importance of ectomycorrhizas lay in their ability to increase the uptake of any nutrient in short supply. This broad view of ectomycorrhizal (ECM) function was also espoused by others, who carried out analyses of *P. strobus* (Mitchell *et al.*, 1937; Finn, 1942). However, some contemporary studies (e.g. McComb, 1938; McComb and Griffith, 1946; Stone, 1950) indicated that absorption of P was more enhanced than that of other nutrients. The publication of these findings was followed within a few years by the first commercial production of ^{32}P , which greatly improved the precision with which movement of P could be traced in biological systems. Its availability led to a significant shift of emphasis towards study of P nutrition of ECM plants and studies of ^{32}P uptake were carried out by Kramer and Wilbur (1949), Harley and McCready (1950) and Melin and Nilsson (1950). These set a trend of work on P uptake and nutrition of ectomycorrhizas which was performed almost to the exclusion of work on uptake of the other elements until the 1980s (see Harley and Smith, 1983).

While these pioneers laid the foundation for much elegant research on the uptake of P by whole plants, the broader view of ECM function espoused by Hatch (1937) and by Harley and Smith (1983) is the one that more closely reflects the cumulative results of research. There are, as discussed in Chapter 15, many circumstances in nature where P deficiency is clearly the primary limitation on productivity of plants

Table 10.1 Growth and specific nutrient uptake of nitrogen, phosphorus, potassium by *Pinus strobus* seedlings.

Degree of mycorrhizal infection	Dry weight	Root weight	Nitrogen		Phosphorus		Potassium	
	(mg)	(mg)	T	SA	T	SA	T	SA
Mycorrhizal (+)	448	180	5.39	0.030	0.849	0.0047	3.47	0.019
	361	170	4.62	0.027	0.729	0.0042	2.57	0.015
Uninfected (0)	300	174	3.16	0.013	0.229	0.0013	1.04	0.006
	361	182	2.51	0.018	0.268	0.0015	1.94	0.011
	301	152	2.40	0.016	0.211	0.0014	1.17	0.008

Data from Hatch (1937). T, total absorbed mg. SA, specific absorption mg/mg root dry weight.

and in which, therefore, ECM colonization may be of particular importance for P nutrition. These are the systems in which further work on P nutrition is still necessary. In those even more widespread areas where elements other than P limit plant growth, work on these nutrients should be emphasized. Whereas the balance has been redressed in this regard in the case of N (see Chapter 9), until recently, relatively few have followed Hatch in considering the possible role of ECM colonization in the capture of K and analyses of the other nutritionally important base cations, magnesium (Mg) or calcium (Ca), have been equally scarce. Fortunately, as described later in this chapter, there has now been some upsurge of interest in these elements. Increasing use of the stable isotopes of potassium (⁴¹K), magnesium (²⁵Mg) and calcium (⁴⁴Ca) to trace the movement of these ions through extraradical mycelia and across the ECM mantle is providing new insights.

Uptake of P by excised ECM and non-mycorrhizal roots

Much of the detailed experimental work on the mechanism of uptake of nutrients, particularly P, by ectomycorrhizas has been done with excised roots in which both the external mycelial system in the soil and the throughput of water are eliminated. The overwhelming reason for using excised mycorrhizas for investigating certain aspects of mycorrhizal physiology is that uniform samples can be obtained for studying specific aspects of the uptake processes. Whole root systems are composed of mycorrhizas, uncolonized primary roots and secondarily thickened axes in different proportions so that it is almost impossible to conclude anything about detailed mechanisms in ECM roots from them. It is easy to obtain large numbers of similar ECM roots from the surface layers of forest soil, wash and prepare them with no great effort. Of course, excised mycorrhizas and non-mycorrhizal roots can also be obtained from aseptically grown plants in the laboratory, but the labour of providing them in sufficient quantity for experimental work on a large scale is daunting.

The criticisms levelled at the use of excised mycorrhizas are those applicable to all experiments with excised roots, i.e. that the transpiration stream is eliminated and the tissue may become starved of organic C during the experiment. In the case of ectomycorrhizas, there is the additional problem that the root is detached from what is increasingly seen to be the critical absorbing system, the extraradical mycelium. Setting these problems aside for the moment, the factors that affect the rate of absorption of nutrients by excised ectomycorrhizas are similar to those which affect

the rate of absorption by most plant material including roots. This was an important contribution from the work of Harley's group using *Fagus* roots most probably colonized by *Lactarius subdulcis* (see Colour Plate 6.1b) (e.g. Harley and McCready, 1952a, 1952b; Harley *et al.*, 1953, 1954, 1956; Harley and Jennings, 1958; Harley and Wilson, 1959; Carrodus, 1966 and see Harley and Smith, 1983) and served to focus attention on the fact that nutrient absorption by mycorrhizas was more relevant to the natural situation than work with non-colonized roots. In the ensuing account, the aim will be to consider the manner in which the absorption physiology of ectomycorrhizas differs from that of non-colonized roots. The work with excised mycorrhizas was predicated on the idea that the mantle tissue that covers the root resembled the extraradical mycelium and that uptake characteristics of the mantle could be extrapolated in developing ideas of nutrient uptake by hyphae from soil. As we have seen from discussions of the changes in enzyme activities in mycelium and mantle (see Chapter 6), this assumption must be viewed very much as a first approximation.

The development of the fungal mantle, as well as the activity of the extraradical mycelium, has important impacts on the duration of active absorption by a root system. The most active non-colonized root apices differ from mycorrhizas in that they are dividing and growing. It is well known that rate of uptake in the tip region of a growing root is much greater than that in the region behind it. This is not generally true of ECM apices. McCready (unpublished) found that the uptake of P by *Fagus* mycorrhizas did not change greatly over distances of 12 cm. However, the rate of absorption of ECM and non-mycorrhizal roots may be very different as shown in Table 10.2 (Harley and McCready, 1950; Bowen and Theodorou, 1967). The differences were also emphasized by the autoradiographs of Kramer and Wilbur (1949) and also of Harley and McCready (1950) that show intense P accumulation in the mycorrhizas and in the extreme apices of non-colonized roots, but not elsewhere. Bowen (1968) scanned the long roots of *P. radiata* with a Geiger counter, following feeding of ^{32}P and showed that, whereas the most active region of accumulation in uncolonized roots was at the apex and in the positions of the apices of developing short roots, that of long root bearing mycorrhizas was at its apex and more particularly at the positions of the ECM rootlets.

Harley and McCready (1952a) showed, using ^{32}P , that the exposure of excised roots to a bathing solution resulted in greater accumulation of P in the fungal mantle. By dissecting the fungal layer from the plant tissue (the core; see Figure 8.2c), they were able to estimate the relative quantities accumulated in the two symbionts. This method was later used for more detailed analyses, in particular of C (see Chapter 8) as well as P fractions and other nutrients. In the case of P, about 90% of that absorbed was found in the sheath after uptake from low concentrations. This observation has since been often confirmed. Harley and McCready (1952a) verified that the accumulation was not dependent upon, nor influenced by, excision. They compared the distribution of P between mantle and root (the core) tissues in mycorrhizas attached to adult trees in the forest and those detached. Comparisons were made on three occasions: when the trees were leafless, when developing their leaves and when in full leaf. On all occasions, there was a great accumulation of P in the sheath (Table 10.3), although the extent to which P was absorbed within rather than adsorbed onto the surface of the sheath was not determined.

These observations of Harley and McCready (1952a) extended only up to July and suggested little seasonal variation in the rate of removal of P from solution. It

Table 10.2 Comparative uptake rates of phosphate by excised mycorrhizas and non-mycorrhizal roots.

Authors and host	Fully infected	Uninfected	Sheath poorly developed
Harley and McCready (1950) <i>Fagus sylvatica</i>	5.18	0.88	4.76
	6.68	0.75	1.20
	1.97	0.42	0.62
	2.72	0.61	—
	1.69	0.72	2.13
Bowen and Theodorou (1967) <i>Pinus radiata</i>	7.5	3.5	—
	15.5		
	15.0	5.5	5.5

The values for each host are relative to one another.

Table 10.3 Estimates of the proportion of phosphorus which accumulates in the fungal mantle of *Fagus* mycorrhizas when attached to the parent root system or when excised. Experiments in Bagley Wood with roots of adult trees at three seasons. Mycorrhizas in aerated phosphate solution pH 5.5 at ambient temperature.

Condition of mycorrhizas	Attached			Detached				
	31 March	11 May	23 July	31 March	11 May	23 July		
mM KH ₂ ³² PO ₄	0.074	0.32	0.16	1.6	0.074	0.32	0.16	1.6
Mean percentage in sheath	88	88	90	87	91	89	85	91
Range	74–96	83–94	89–94	86–93	85–96	79–93	83–89	91–93

Data from Harley and McCready (1952a).

has since been shown that the ectomycorrhizal roots of *Abies balsamea* (Langlois and Fortin, 1984) and *Picea sitchensis* show a distinct seasonality in their ability to absorb P, maximum rates being achieved in the late summer or autumn after completion of shoot extension growth. Further, it must be borne in mind that the patterns of accumulation described by Harley and McCready (1952a, 1952b) were in all cases obtained using ECM roots from which the extraradical mycelium had become detached. When the kinetics of P absorption by these roots are compared with those seen in intact mycorrhizal systems (van Tichelen and Colpaert, 2000), it is observed that the rates achieved by the excised roots are considerably lower (Figure 10.1 and see below). While differences of experimental conditions and in the physiology of the mycobionts involved must contribute to such effects, there is every likelihood that the removal of the mycelial network strongly influences the observed rates of P uptake.

The results of Harley and McCready (1952a, 1952b) were obtained by dissecting roots following a period of exposure to solutions containing orthophosphate. Two features must be noted. First, as external solution is applied to the mantle, accumulation can appear to take place as P is adsorbed onto its surface or as it passes through the fungal tissue to the core within. Second, there may be a real accumulation depending upon the species of fungus and its activity. Garrec and Gay (1978) analysed the mycorrhizas of *Pinus halepensis* using an electron probe and concluded that P is mainly accumulated in the fungal mantle and Hartig net region and is

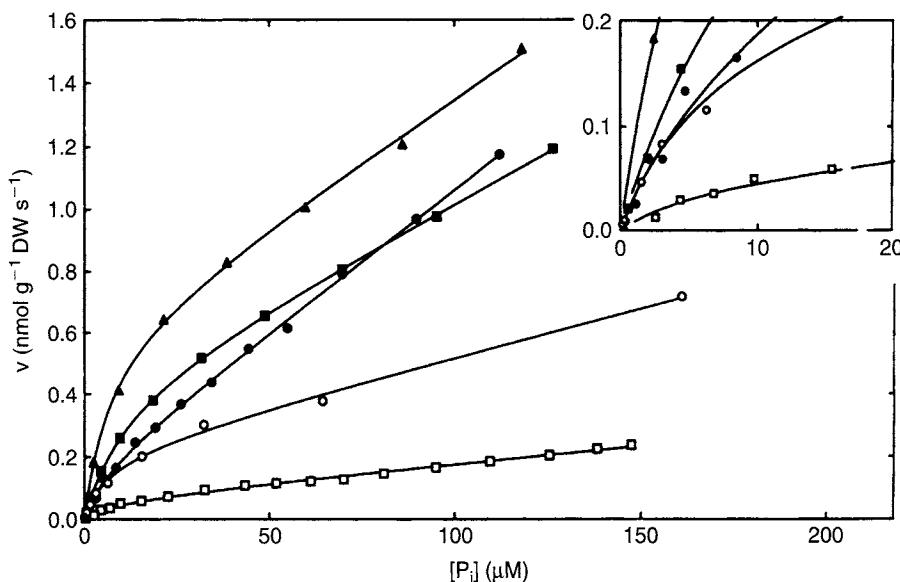


Figure 10.1 Representative Michaelis-Menten plot showing the net orthophosphate (P_i) uptake in intact mycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings as a function of solution P_i concentrations (0.2–160 μM). The inset figure shows the relationship at the lower P_i concentrations most representative for soil solution. Measurements were performed 9 weeks after inoculation and data points were fitted to a two-phase Michaelis-Menten equation. ▲ *Paxillus involutus*; ■ *Suillus bovinus*; ● *Thelephora terrestris*; □ non-mycorrhizal. For comparison data obtained with excised *Fagus sylvatica*–*Lactarius subdulcis* mycorrhiza (○) are also plotted (Harley and McCready, 1952b; with permission). From van Tichelen and Colpaert (2000).

lower in the plant tissue. Since the mantle, whether it accumulates nutrients permanently or acts as a temporary store, separates the plant tissue from the soil, the mechanism of the passage of substances through it and factors affecting their rate of transfer to the plant require investigation.

The absorption of orthophosphate into excised mycorrhizas of *Fagus* results in its immediate incorporation into nucleotides and sugars (Harley and Loughman, 1963). Both separated fungal mantle and plant core exhibit rapid incorporation of applied ^{32}P but, in intact mycorrhizas, the core tissue receives less P and appears more sluggish in incorporating orthophosphate into other compounds, with only about 10–20% of that absorbed passing steadily into the core tissues through the mantle. By studying the time course of esterification of the P entering the root core, Harley and Loughman were able to show that the labelling of orthophosphate represented 100% of the radioactivity in that tissue initially and that proportion fell, as nucleotides, sugar phosphates and other fractions became labelled. Since both the mantle and the plant tissues showed the same labelling pattern of soluble P compounds if allowed separately to absorb orthophosphate from radioactive solutions, it was concluded that in the intact excised mycorrhizas orthophosphate was the form which passed from the mantle to the plant when low concentrations were applied externally.

Harley and McCready (1952b) and Harley *et al.* (1958) studied the possible routes by which P might pass through the mantle from the external solution to the plant.

They showed first that the mantle prevented the plant from absorbing P at its maximum possible rate, except when very high concentrations were present in the solution. They concluded that from low P concentrations, such as might be expected in the soil, diffusive movement through the mantle did not take place at a significant rate. It might, however, occur at concentrations above about 1 mM, which are totally unrealistic ecologically. The P passing through the sheath to the plant root did not equilibrate with a large part of the P in the fungus. If it did so, as Harley *et al.* (1954) showed, a lag phase in the arrival of P in the core tissue would be expected and the quantity of P in the pathway to the core would be related to the length of the lag phase. Using low external P concentrations, the lag phase was exceedingly short, so that the quantity of P in the mantle with which the passing inorganic P equilibrated was small – of the order of 0.017 µg P per 100 mg dry weight of mycorrhizas. This is extremely low compared with the amount of P present in the sheath and it was concluded that orthophosphate is incorporated first into the metabolic pools of the fungal symplast in the sheath and that these constitute a small proportion of the total P, as they do in other roots (Crossett and Loughman, 1966).

Following uptake, ECM fungi, both in culture and in symbiosis, synthesize polyphosphate (polyP), which is stored in the vacuoles, thus maintaining relatively low cytoplasmic Pi concentrations. In ectomycorrhizas, some of the P in the extra-radical mycelium and the sheath is certainly present as polyP. This was pointed out by Ashford *et al.* (1975) using *Eucalyptus* and subsequently shown to be true of ectomycorrhizal *Pinus radiata*, of the arbutoid mycorrhizas of *Arbutus unedo* and of the arbuscular mycorrhizas of *Liquidambar styraciflua* (Ling Lee *et al.*, 1975). Using the same cytochemical methods as Ling Lee *et al.* (1975), Chilvers and Harley (1980) described particles believed to be polyP in the sheath of *Fagus* mycorrhizas. The number and size of particles increased during P absorption at rates similar to the rate of absorption of P from similar concentrations and similar factors affected their formation as affected P uptake. At this stage it was assumed that polyP was stored as long-chain, insoluble granules, a view that has subsequently been re-evaluated (see below). The formation of polyP in *Fagus* mycorrhizas was further examined by Harley and McCready (1981) using the method of extraction and precipitation described by Aitchison and Butt (1973). Assuming that there was little hydrolysis in the extraction and that the precipitation with BaCl₂ was complete, it was concluded that a large amount of the P accumulated in the mantle tissue is polyP. More recent work has confirmed the location of the polyP to be in the fungal vacuoles (Ashford *et al.*, 1994; Ashford and Orlovich, 1994; Gerlitz and Gerlitz, 1997; Bücking and Heyser, 1999), the pH values of which are inherently low (Rost *et al.*, 1995). Much early work suggested that the polyP occurred as granules, stabilized by Ca²⁺ or arginine (e.g. Ashford *et al.*, 1975; Ling-Lee *et al.*, 1975). Hypotheses were formulated involving these polyP granules in both storage and translocation in fungal hyphae and in the mantle tissues of ectomycorrhizas. More recently, it has been suggested that the granules may be artefacts of specimen preparation (Orlovich and Ashford, 1993; see Figure 10.2) and hypotheses depending on their behaviour must be re-evaluated.

There were early indications from NMR spectroscopy (BC Loughman, personal communication, see Harley and Smith, 1983) that at least part of the polyP in the fungal mantle of ECM of *Fagus* occurs as relatively short-chain molecules. The data of Martin *et al.* (1985), again using ³¹P NMR, also indicated a large, soluble polyP fraction in the mycelium of *Cenococcum geophilum* and *Hebeloma crustuliniforme*.

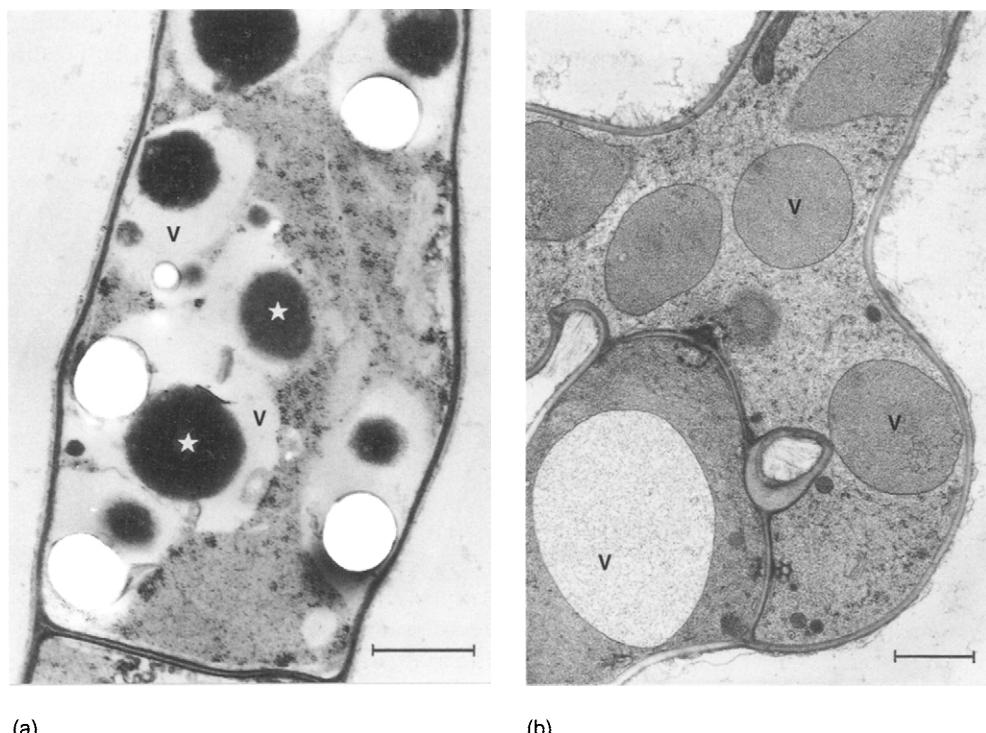


Figure 10.2 Effect of fixation methods on the preservation and distribution of polyphosphate in *Pisolithus tinctorius*. (a) Transmission electron micrograph of a glutaraldehyde-fixed hypha embedded in Spurr's resin and triple stained. Note the spherical opaque granules (★) in vacuoles (V). (b) Transmission electron micrograph of a stained ultrathin section of part of a freeze-substituted hypha near a clamp connection. The vacuoles (V) contain electron-opaque material which is evenly dispersed. There are no discrete granules. Bars = 1 µm. From Orlovich and Ashford (1993), with permission.

PolyP present either in chains longer than about 70 orthophosphate residues or as insoluble precipitated material (granules) cannot be detected by NMR spectroscopy. Importantly, the behaviour of polyP in the fungal mycelium revealed by NMR was similar to that shown by counting the (possibly artefactual) granules in excised mycorrhizal roots (Chilvers and Harley, 1980; Strullu *et al.*, 1981a, 1981b, 1982). PolyP accumulation varied with different stages of growth, being low when growth was rapid in young mycelia and linear in the early and late stages of the stationary phase when P in the medium was relatively abundant compared with N. When the mycelium was transferred to low P medium, the NMR spectra indicated mobilization of polyP, rapidly in *H. crustuliniforme* and more slowly in *C. geophilum*. Martin *et al.* (1985) discussed the apparently conflicting evidence on the form of polyP, concluding that the spin-lattice relaxation times of the ^{31}P nuclei in both fungi were consistent with a single pool of relatively fluid poly-P, possibly in the form of 'macromolecular aggregates'. They certainly found no evidence for granules and considered the investigation of purified granules by NMR spectroscopy to be 'particularly urgent'! Orlovich and Ashford (1993) obtained data from anhydrous freeze

substituted material, indicating that, in *P. tinctorius*, polyP is uniformly distributed in the fungal vacuoles and is stabilized by K⁺ (Figure 10.3). PolyP of about 15 orthophosphate units was extracted from the mycelium and identified by chromatography, gel electrophoresis and ³¹P NMR. This investigation also illustrated the formation of granules stabilized by Ca²⁺ during chemical fixation, explaining the widespread observation of these 'structures'. However, X-ray microanalytical analysis of thin

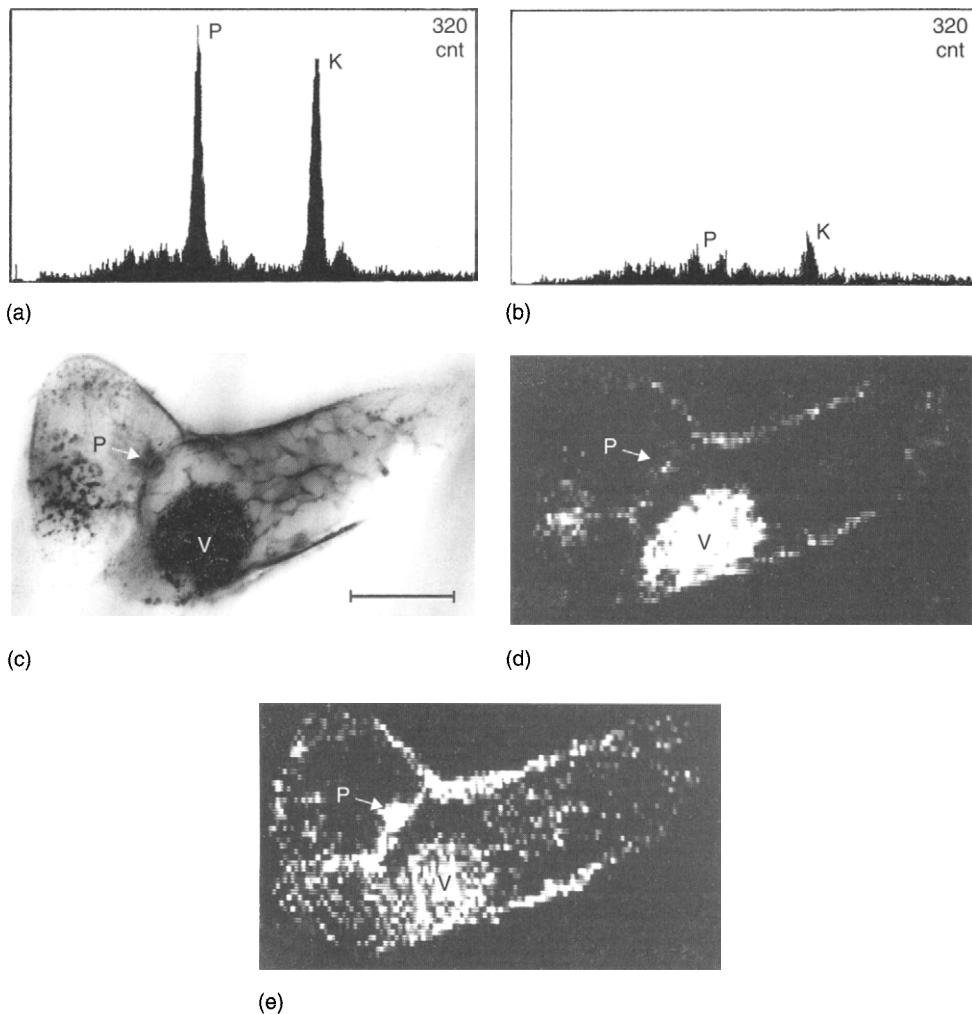


Figure 10.3 Distribution of P and K in a hypha of *Pisolithus tinctorius*. (a) and (b) Energy-dispersive X-ray spectra from the analysis of an unstained, freeze-substituted hypha cut dry at 1 µm. The full vertical scale, measured in X-ray counts, is at the top right of each spectrum. Vacuole showing large peaks for P and K shown in (a) and cytoplasm, showing relatively small peaks for P and K, shown in (b). (c) Transmission electron micrograph of a hypha near a clamp connection. Note the large vacuole (V). Bar = 2 µm. (d) and (e) X-ray maps of the hypha in (c), showing the similar distribution of P in (d) and K in (e) in the vacuole. From Orlovich and Ashford (1993), with permission.

sections of tissues prepared without such fixation (Bücking and Heyser, 1999) indicates that polyP granules are visible in the hyphae of ECM fungi. The matter of the state (fluid or solid) in which polyP occurs is therefore still a matter for debate.

PolyP is located in fungal vacuoles and in the tubular cysternae of ECM fungi (Orlovich and Ashford, 1993; Ashford *et al.*, 1994), all of which compartments are acidic. In consequence, the polyP carries a strong negative charge which must be balanced by association with cations. Positively charged nitrogen compounds, such as the basic amino acid arginine, may play a role as neutralizing agents, but micro-analytical studies have provided evidence of the direct association of vacuolar P with K⁺ and Mg²⁺ ions (Orlovich and Ashford, 1993; Bücking and Heyser, 1999).

Ashford (1998) has hypothesized that the fungal vacuoles occur as tubular systems through which the longitudinal translocation of polyP takes place (Figure 10.4). If this is the case, strong coupling of polyP with K and Mg would inevitably involve co-transport of these cations with P in the direction of the plant (see below). The compartmentation of polyP would enable a great part of the P in the mantle to be separated from the labile P that can move to the plant tissue.

The mechanisms of transfer of P from the ECM partner across the interfacial apoplast into the plant roots continues to be a subject of experiment. Harley and Loughman (1963), through short-term labelling experiments with excised roots of *Fagus*, showed that ³²P-labelled orthophosphate passed from fungus to host. The electrochemical potential difference between the Hartig net and apoplast will favour passive efflux from the fungus into the apoplast. However, rates of P loss by fungal hyphae are generally rather low, so that mechanisms which promote efflux and, at the same time, reduce retrieval or reabsorption, seem likely to operate at the interface. Uptake by the plant symbiont must be active, as the cells accumulate P against a strong electrochemical potential gradient. Recent work has identified transporter genes that may be implicated in P uptake by the fungal partner from soil, as well as plant genes potentially involved in transfer at the symbiotic interface. Several low- and high-affinity transporters have been identified in both *Laccaria bicolor* and *Hebeloma cylindrosporum*, but it is not yet known what roles they may play in P uptake from soil or redistribution in fungal tissues. On the plant side, 13 homologues of high-affinity Pi transporters in the Pht1 family have been identified in *Populus*. One of these is preferentially expressed in ECM root tips. It is presumed, but not yet demonstrated, that expression will be localized in the plant cells adjacent to the Hartig net (Martin, 2007). There have been suggestions that the supply of P to plants may be quantitatively linked to loss of sugars to the interfacial apoplast. However, as yet no mechanisms that relate influx of P to efflux of sugars have been identified.

Bücking and Heyser (2000) showed that the translocation of P across the ECM interface was regulated by the orthophosphate concentration in the cytoplasm of the Hartig net and by the efflux into the interfacial apoplast. These authors stress that the relationships between P supply to the mycorrhiza and transfer to the host are strongly dependent upon fungal species. Whereas in mycorrhizas formed by *Suillus bovinus* an increased P supply to *Pinus sylvestris* roots had no effect on translocation of the element across the ECM interface to the plant, a supply-dependent effect was observed in mycorrhizas formed by *Pisolithus tinctorius*. Clearly, caution must be exercised when attempting to generalize from patterns seen in one mycorrhizal system to all others.

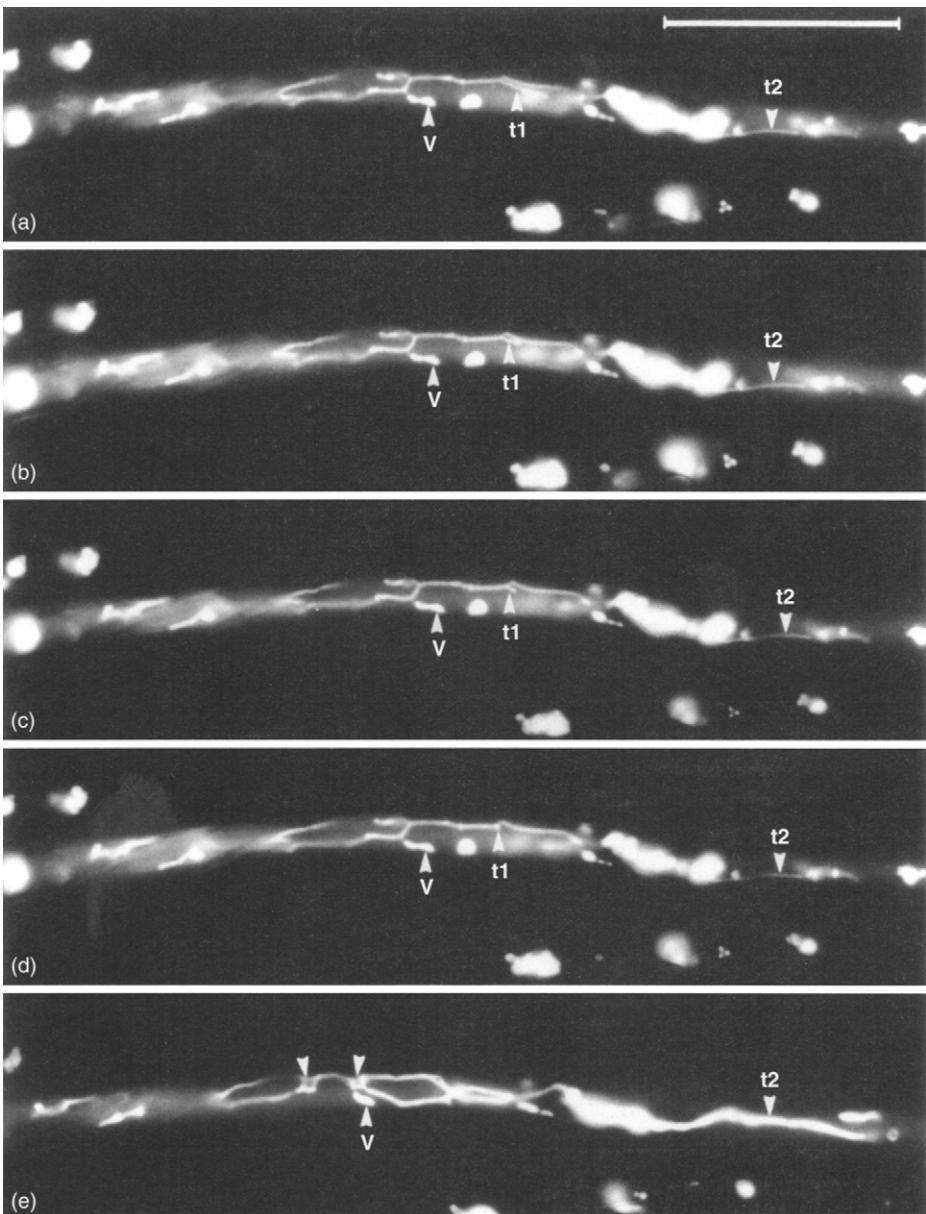


Figure 10.4 Time-lapse photomicrographs (a)–(e) taken at 4 second intervals in the same focal plane showing movements of the tubular reticulum in the apical cell of a hypha of *Pisolithus tinctorius* following loading with carboxyfluorescein. A vacuole (v) remains connected to the reticulum via a narrow fluorescent bridge throughout. The sequence shows consecutive frames of the retraction of a tubule (t1) that constitutes a branch of the reticulum. At some branch point (arrowheads) a plaque-like ring structure is seen surrounding a non-fluorescent area. The tubule (t2), which remained very fine initially (a) to (d) becomes obliterated (e) by a broader tubule that is confluent with the fluorescence of the vacuole cluster. From Shepherd et al. (1993b), with permission.

Phosphate absorption in intact plants

Following uptake, the slow transfer in excised roots is consistent with data from experiments on whole plants of *Pinus radiata* (Morrison, 1957a, 1962a), in which seedlings were grown at two levels of P in pots in the greenhouse for 17 weeks. With high P, the non-mycorrhizal plants grew somewhat better than the ECM ones, but the reverse was true with low P. Plants of each kind were then grown with fresh supplies of P, labelled with ^{32}P . In all experiments the movement of ^{32}P to the shoot tip of the non-mycorrhizal plants was rapid at first, but later decreased in rate and almost ceased, but could be increased again by further addition of P to the soil. Movement to the shoot tips of ECM plants, although much slower than the initial rate in non-mycorrhizal plants, continued steadily for weeks and was little affected by further additions of labelled P to the soil. The accumulated ^{32}P in the shoot tips of ECM plants eventually exceeded that of the non-mycorrhizal controls. If ECM and non-mycorrhizal plants were deprived of P after a period of uptake of ^{32}P , radioactivity continued to pass to the shoots of ECM plants for three weeks, but ceased to move to those of non-mycorrhizal plants after only a short period. This behaviour is explicable in terms of the accumulation of P in the fungal mantle, coupled with a steady rate of transfer to the plant when P is available. When P supplies are deficient, mobilization of the stored P in the mantle occurs. This type of study is instructive in terms of distribution of P in the plant, but tells us little about the processes of P capture from soil by the extraradical mycelium.

Stone (1950) compared two samples of seedlings of *P. radiata* with very different development of extraradical hyphae. Those with a more extensive system absorbed ^{32}P from the soil faster and translocated a greater quantity to their needles. Similarly, Melin and Nilsson (1950) showed that ^{32}P orthophosphate fed to the extraradical mycelium of *P. sylvestris* was translocated to the root by the hyphae and thence through the plant to the needles. Skinner and Bowen (1974a, 1974b), using *P. radiata* and *Rhizophagus luteolus*, confirmed the transport of P in ECM rhizomorphs. P absorption by rhizomorphs was inhibited by cyanide and was temperature dependent and subsequent translocation occurred over distances of up to 12 cm. However, there were large differences in extent of mycelial growth in the soil between strains of fungus and between samples of the same fungal isolate in different soil conditions (Skinner and Bowen, 1974b). Experiments such as these emphasize the need for the extent of production of extraradical hyphae and rhizomorphs to be fully described in experiments on the efficacy of different combinations of fungal strain and plant genotype. The differences between the absorptive capabilities of mycobionts has been emphasized in studies using *P. sylvestris* seedlings grown in the non-mycorrhizal condition or with a number of commonly occurring ECM fungi (Colpaert *et al.*, 1999; van Tichelen and Colpaert, 2000). At an external Pi concentration of 10 μM , ECM seedlings colonized by *Thelephora terrestris* and *Paxillus involutus* achieved P uptake rates that were, respectively, 2.5 and 8.7 times higher than those of their non-mycorrhizal counterparts. Positive correlations were found between P uptake rates and the biomass of the external mycelium of each mycobiont, measured by ergosterol assay. In detailed kinetic analyses, van Tichelen and Colpaert (2000) further emphasized the importance of mycobiont effects as determinants of the ability of *P. sylvestris* to capture P. Net P uptake was dependent upon concentration and was governed by Michaelis-Menten kinetics. However, a dual uptake process consisting of

high- and low-affinity systems operating simultaneously was revealed. Representative Michaelis-Menten plots (see Figure 10.1) confirmed the large differences between mycobionts in net uptake of P and again demonstrated the effectiveness of *P. involutus* in this regard. Comparisons of P uptake rates in four mycobionts of *P. sylvestris* with those calculated from the data of Harley and McCready (1952a) for excised *F. sylvatica-Lactarius subdulcis* roots (see Figure 10.1 inset; Table 10.4) indicate that, whereas the high- and low-affinity systems are present in both excised and intact roots, the V_{max} values, as well as the overall P uptake rates, are higher in three of the pine mycobionts.

Finlay and Read (1986b) used autoradiography to examine the uptake of ^{32}P by the extraradical mycelium of *Suillus bovinus* and its transport to seedlings of *Pinus* spp. which were interlinked by the fungus (Figure 10.5a). A seedling of *P. sylvestris* colonized by the fungus was first introduced to an observation chamber containing non-sterile peat and the system was incubated for a period sufficient to enable the mycelium of *S. bovinus* to colonize both the peat and series of previously uncolonized seedlings of *P. contorta*. ^{32}P -orthophosphate fed at about 30 cm from the seedling roots was absorbed by the fungus over a period of 72 h and was translocated throughout the peat and to the ECM roots of all the plants interlinked by the fungus. ^{32}P accumulated in ECM roots in a pattern that would be predicted from the studies of excised roots described above. In some seedlings, onward transfer of ^{32}P to the shoots took place in the same period. Distribution of ^{32}P in the extraradical mycelium in the peat was irregular. It was clear that the rhizomorphs provided the main pathways for long-distance translocation, but there was also directional transport toward the actively growing hyphae at the advancing mycelial front and into patches of dense mycelium (Figure 10.5b).

The extraradical component of ectomycorrhizas is extremely important in colonizing the soil and may play a role similar to that described by arbuscular mycorrhizal (AM) hyphae (see Chapters 5 and 6). However, both the extent of development of

Table 10.4 Kinetic parameters of the high-affinity uptake system in intact mycorrhizal and non-mycorrhizal *P. sylvestris* root systems.

Harvest	Inoculation treatment	No. of plants	No. of points	Goodness of fit (mean) R	Deviation from model (Runs test) P	K_m (μM)	V_{max} (nmol/g/s)
Week 7	<i>Paxillus involutus</i>	3	13	0.998	NS (0.73)	3.5	0.57
	<i>Suillus bovinus</i>	3	15	0.999	NS (0.52)	7.5	0.49
	<i>Thelephora terrestris</i>	3	20	0.997	NS (0.81)	8.7	0.13
Week 9	<i>Paxillus involutus</i>	3	9	0.999	NS (0.89)	5.9	0.62
	<i>Suillus bovinus</i>	3	10	0.999	NS (0.64)	10.2	0.52
	<i>Thelephora terrestris</i>	3	16	0.999	NS (0.81)	7.3	0.15
	Non-mycorrhizal	4	17	0.995	NS (0.11)	12.1	0.08
	<i>Lactarius subdulcis</i>		12	0.998	NS (0.99)	6.4	0.21

Data from van Tichelen and Colpaert (2000). NS, not significant. The K_m and V_{max} values were calculated by iterative fitting of the data to Equation (3) (sum of the two Michaelis-Menten terms); mean and range (between brackets) are shown. The average number of points used for this procedure is provided as well as the number of plants analysed. Kinetic parameters obtained with excised *F. sylvatica-L. subdulcis* mycorrhizas are included for comparison (calculated for the 0.2–160 μM P_i range, from Harley and McCready, 1952b).

the ECM mycelium and the abilities of some of the fungi to utilize sparingly soluble organic P sources are generally greater in ECM than in AM symbiosis (Marschner, 1995; George and Marschner, 1996; see Chapter 5). The hyphae not only extend beyond any zone depleted of nutrients near the surface of the mantle, but also may readily be extended or replaced with a small expense of C and nutrients per unit area of absorbing surface. Furthermore, the hyphae proliferate in microsites in the soil and exploit the resources in them (see Chapter 6). Laboratory studies (Wallander and Nylund, 1992; Ekblad *et al.*, 1995; Jentschke *et al.*, 2001a) have shown that proliferation of the mycelial system of ECM fungi can be greatly stimulated by the addition of P to otherwise P-deficient substrates. These observations, taken in association with those of Häussling and Marschner (1989) showing a linear relationship between the lengths of extraradical mycelial systems and phosphatase activity (Figure 10.6), are indicative of a close coupling between P supply to the plant and the extent of foraging for the element in soil.

Rousseau *et al.* (1994) quantified the difference in potential absorbing surface area between seedlings of *P. taeda* colonized by the fungi *Pisolithus tinctorius* and

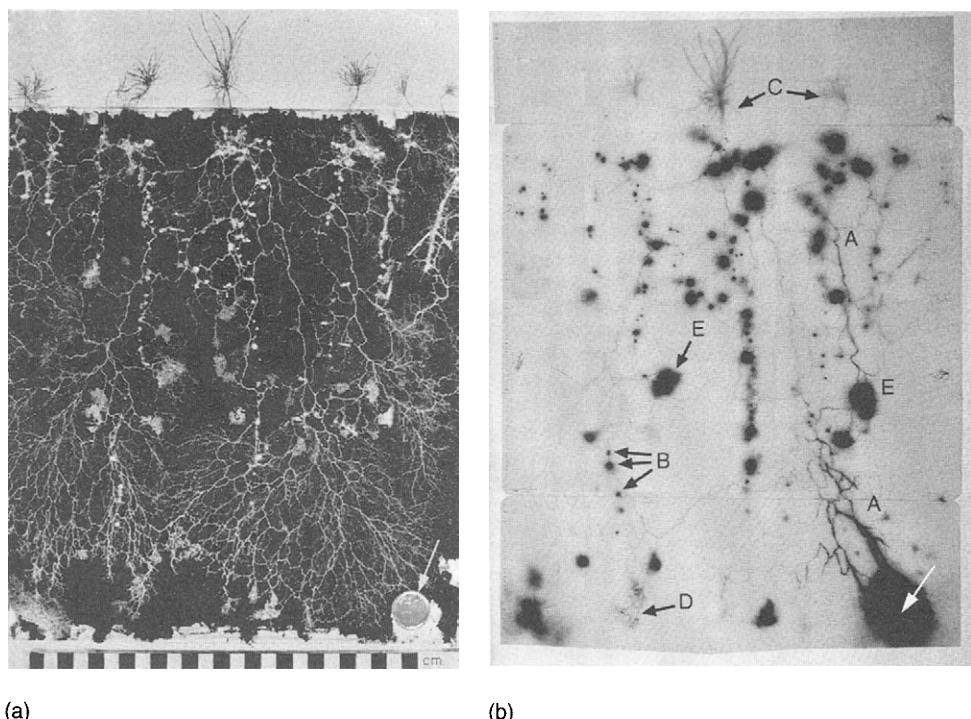


Figure 10.5 Transport of ^{32}P through the extraradical mycelium of *Suillus bovinus*, linked to seedlings of *Pinus sylvestris* and *P. contorta*. (a) Root observation chamber showing the mycelial connections between the plants and the site of feeding with ^{32}P in half-strength Melin-Norkrans medium (arrowed). (b) Autoradiograph of the same chamber showing the distribution of ^{32}P after 82 hours. Label has accumulated in the rhizomorphs (A), mycorrhizal roots (B) and the shoots (C). There is also some accumulation in the advancing mycelial front (D). From Finlay and Read (1986b), with permission.

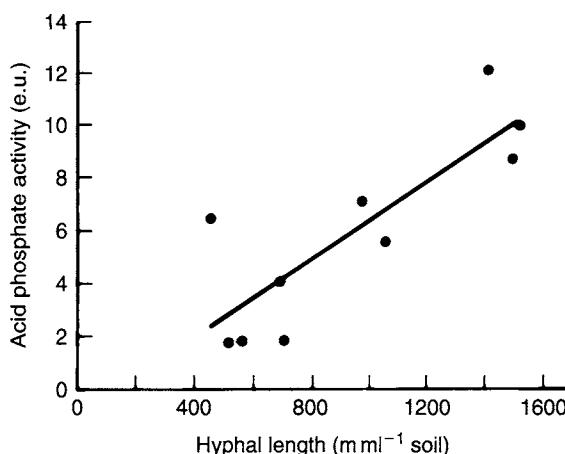


Figure 10.6 The relationship between length of external hyphae and phosphatase activity (in enzymes units, EU) in the humus layer in which mycorrhizal plants of *Picea abies* were growing. From Häussling and Marschner (1989), with permission.

Cenococcum geophilum and those that grew in the non-mycorrhizal condition. They also examined the differential effect of the two fungi on P uptake by the plants (Table 10.5). Whereas *P. tinctorius* stimulated some increase in branching of root tips and hence surface area of fine roots, *C. geophilum* did not do so. The major impact of the fungi on the area available for absorption was provided by the extraradical hyphae which led to an increase of approximately 40-fold in the case of *P. tinctorius* and 25-fold for *C. geophilum* in relation to the non-colonized controls. The greater lengths, and consequently surface areas, of the mycelia were associated with significant increases in P uptake by both fungi and with greater shoot weight in the case of plants ECM with *P. tinctorius*. While there appears to be a correlation here between hyphal development and P uptake, studies of this kind do not conclusively demonstrate a causal relationship between the two.

To determine such a relationship, P uptake must be measured in terms of inflow (uptake per unit length of root per unit time) or specific uptake rate (uptake per unit weight per unit time). That the effects of the fungus can be large has been appreciated for some time and, as Bowen (1973) pointed out, estimates of the specific uptake rate can be calculated from the data of Hatch and others (see Table 10.1) and the results demonstrate the greater uptake that follows from the improved exploitation of the soil with respect to poorly mobile P, even though the values must be underestimates because the contents of nutrients in the seed are not known.

Two studies, one of *Eucalyptus pilularis* colonized by a fungus of uncertain identity but probably *Cenococcum geophilum* (Heinrich and Patrick, 1986) and another of *Salix viminalis* colonized by *Thelephora terrestris* (Jones *et al.*, 1991) have shown unambiguously that ECM colonization of intact root systems increases P inflow significantly. Heinrich and Patrick (1986) established relationships between numbers of *Cenococcum*-type ectomycorrhizas and both seedling dry weight (Figure 10.7a) and total seedling P content (Figure 10.7b). Significant correlations were observed for both relationships. In the case of *Salix*, the inflows of P to ECM roots were almost three times higher than to those that were uncolonized. The substantial increase in

Table 10.5 A comparison of plant and fungal parameters for *Pinus taeda* seedlings colonized by *Pisolithus tinctorius* (Pt), *Cenococcum geophilum* (Cg) or left uncolonized (control).

	Pt	Cg	Control	P*
Mycorrhizal infection (%)	69.5 a	66.5 a	0.0 b	<0.0001
Shoot weight (g)	1.09 a	0.830 b	0.710 b	0.015
Foliar P conc. (g)	0.066 a	0.043 b	0.034 b	<0.0001
Shoot P content (mg)	0.669 a	0.340 b	0.238 c	<0.0001
Fine-root diameter (mm)	0.477 b	0.573 c	0.299 a	<0.0001
Root-tip ratio	3.72 b	1.39 a	1.55 a	<0.0001
Area fine-root (mm ²)	4.02 b	1.49 a	1.30 a	<0.0001
Area (mm ² /g soil)				
Hyphae	33.8 a	28.1 a	1.50 b	<0.0001
Rhizomorphs	13.6 a	0.00 b	0.00 b	0.0012
Total	47.4 a	28.1 b	1.50 c	<0.0001
Length (m/g soil)				
Hyphae	6.42 a	2.80 b	0.28 c	<0.0001
Rhizomorphs	0.36 a	0.00 b	0.00 b	0.0011
Total	6.78 a	2.80 b	0.28 c	<0.0001
Dry weight (μg/g soil)				
Hyphae	4.98 a	7.85 b	0.22 c	<0.0001
Rhizomorphs	14.3 a	0.00 b	0.00 c	<0.0001
Total	19.3 a	7.85 b	0.22 c	<0.0001
Hyphal diameter (μm)	1.60 a	3.18 b	—	<0.0001

Data from Rousseau *et al.* (1994). Values within a row having the same letter are not statistically different (Duncan's, $P < 0.05$). P, Probability values from one-way ANOVA between inoculation treatments.

P supply supported a twofold increase of growth (Jones *et al.*, 1991). Subsequently, Jones *et al.* (1998) determined the relative abilities of ECM and AM fungi to enhance P uptake rates of *Eucalyptus coccifera*. Although the two ECM symbionts, *T. terrestris* and *Laccaria bicolor*, differed in their effectiveness in P acquisition for the plant, they were both significantly more effective than the two species of AM fungi, *G. caledonium* and *G. mosseae*. Overall, P inflows to ECM plants were increased by 3.8 times and to AM plants by between 2.0 and 2.7 times, relative to those seen in the non-mycorrhizal condition.

There are studies indicating that hyphal development in soil can be a poor indicator of mycorrhizal effectiveness. Thomson *et al.* (1994) found that, while those fungi which were most effective in increasing the uptake of P and growth in *Eucalyptus globulus* were also those that colonized the roots most extensively, P uptake correlated poorly with hyphal length. Thus, the fungus most effective in increasing plant growth, *Descoclea maculata*, formed the smallest amount of external hyphae per metre of colonized root, while isolates of *L. laccata* developed more external hyphae per metre of root than other fungi, without any apparent additional benefit to the plant. Observations such as these indicate the need for caution in generalizing about the role of hyphal length in P uptake. Clearly, other factors, among which the viability and physiological characteristics of the mycelium and the compatibility between fungus and plant, as well as in exploring the soil for new plant sources of organic C may be important and should be taken into account.

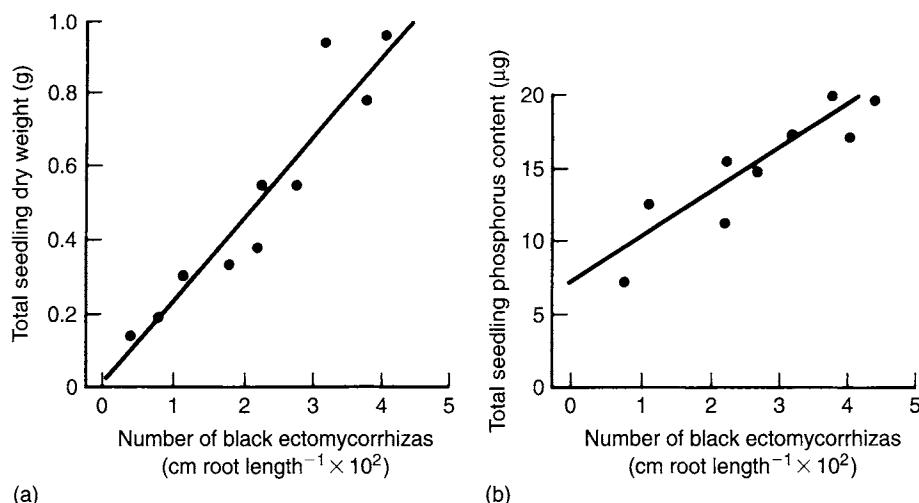


Figure 10.7 Growth and P uptake of *Eucalyptus pilularis* colonized by *Cenococcum geophilum* 176 days after sowing. (a) Relationship between number of black ectomycorrhizas and total seedling dry weight. (b) Relationship between number of black ectomycorrhizas and phosphate content of the seedlings. From Heinrich and Patrick (1986), with permission.

A number of other studies of responses of colonized and uncolonized plants to changing concentrations of P have now been published. That of Boughey *et al.* (1990) on *Eucalyptus* has already been described (see Chapter 8). *Pinus taeda* responded positively to inoculation with four fungi (*Pisolithus tinctorius*, *Rhizopogon roseolus*, *Scleroderma aurantium* and *T. terrestris*) when growing in Piedmont forest soil at all P concentrations supplied, relative to uninoculated controls after ten months (Ford *et al.*, 1985). Responses to *S. aurantium* were much greater than to the other fungi. This was a pot study in a glasshouse which, considered in isolation, could be taken to indicate that similar responses to P and inoculation might be expected in the field. However, the authors point out that attempts to improve growth of *P. taeda* in plantations established on these soils by application of P fertilizers had failed because N was in fact the limiting nutrient in the field. The growth responses to P achieved in pots occurred because N limitation had been removed by N fertilization at the start of the experiment. The experiment was thus instructive in terms of the potential of ECM colonization to improve growth, but also emphasizes that the significance of the roles of the fungi will be strongly influenced by field conditions (see Chapter 17).

Sources and mobilization of P in soil

Some discussion of the nature and availability of P sources in soil has been provided in Chapter 5. A large part of the P contained in the surface horizons of forest soils, where most ectomycorrhizas are localized, is present in organic forms (Dalal, 1977; Harrison, 1983). These can occur as phosphomonoesters such as inositol hexaphosphate, often referred to as 'phytate', or as phosphodiesters, among which nucleic acids and phospholipids are likely to be important. In this context the recent indications that 'phytate' may have been misidentified as a major organic P source in soil should be borne in mind and the relevance of studies with pure phytate questioned

(Smernik and Dougherty, 2007, see Chapter 5). Some of the monoesters, though important constituents of living cells, may have only a short life span in soil because endogenous phosphomonoesterases will attack them in the course of cell breakdown (see Beever and Burns, 1980). Others are clearly more resistant to breakdown, since a significant proportion of the organic P in acidic soils may be present as inositol penta- and hexa-phosphates (Cosgrove, 1967; McKercher and Anderson, 1968). The phosphomonoesterases are easily studied and have been detected in most ECM fungi but, in future, it will be important to investigate other (hitherto neglected) enzymes that can degrade organic P.

Evidence for the ability of ECM fungi to produce phosphomonoesterase has taken two forms, one indirect involving studies of growth on phytate supplied as sole P source and the other direct and dependent upon measurement of phosphatase activity using paranitrophenyl phosphate (PNPP) as the substrate. Growth of *Suillus granulatus*, *S. luteus*, *Cenococcum geophilum* and *Rhizopogon roseolus* takes place with phytates of Ca and P as sole sources of P (Theodorou, 1968), though it was shown that Fe phytate, likely to be of greater quantitative significance in acid organic soil, was little used. *R. luteolus* was shown to produce two types of phytase (Theodorou, 1971). Bartlett and Lewis (1973) examined the surface phosphatase activity of mycorrhizas of *Fagus* and also showed the presence of more than one phosphatase, because the activity had a double pH optimum and hydrolysed a range of P compounds including inorganic pyrophosphates and organic compounds especially inositol phosphates. They emphasized that phosphatase of such an activity on the surface of the fungal component of ectomycorrhizas might result in the immediate recycling of the phosphates present in the fallen litter back into the mycorrhizal system. Williamson and Alexander (1975) also examined *Fagus* mycorrhizas. They found that acid phosphatase was present throughout the fungal tissue and was not associated with contaminating microflora to any significant extent. They agreed with Bartlett and Lewis that more than one phosphatase enzyme was present and that each had different characteristics. Alexander and Hardy (1981) showed that mycorrhizas of *Picea sitchensis* possessed surface phosphatase activity that was inversely correlated with the concentration of extractable inorganic P in the soil. In this respect, the work is reminiscent of that of Calleja *et al.* (1980) who showed that the phosphatase activities of four species of ECM fungi were higher in the absence of soluble P in the culture medium. The effects of such environmental variables as pH, temperature and substrate concentration on the activities of acid phosphatases have now been examined in a number of ECM fungi (Antibus *et al.*, 1986) and it has again been shown (Antibus *et al.*, 1992) that enzyme activity and phytate utilization are greatest at low concentration of inorganic P. Colpaert *et al.* (1997) could find no evidence of phytase activity in the mycelia of *Thelephora terrestris* or *S. bovinus* symbiotic with *P. sylvestris* when they were grown in the presence of phytate. Further, the fungi were unable to obtain P from phytate fixed on HPLC resin. These workers were unable to find support for the hypothesis that phytate is a useful P source for ECM plants.

Dinnelaker and Marschner (1992) demonstrated that phosphomonoesterase activity was greater in the ECM roots of spruce and in the rhizomorphs of *T. terrestris* than in non-mycorrhizal roots. The localization of these enzyme activities on the surfaces of fungal hyphae necessitates close juxtaposition with appropriate P sources. The preferential proliferation of the extraradical mycelium in P-enriched substrates, whether they be hyphal mats (Griffiths and Caldwell, 1992) or mesh

bags (Hagerberg *et al.*, 2003) in the field, or litter 'patches' in microcosms (Bending and Read, 1995a; Perez-Moreno and Read, 2000) (see Figure 6.27a, b), will facilitate this physical proximity.

To date, studies of what are likely to be the more important phosphodiesterase activities have been few. Griffiths and Caldwell (1992) found that the mat-forming ectomycorrhizal fungi *Gautieria monticola* and *Hysterangium gardneri*, together with an unidentified *Chondrogaster* species, were capable of hydrolysing the major phosphodiester RNA and it has been shown by Leake (unpublished) that *S. bovinus*, one of the fungi known to produce dense mycelial patches (see Figure 6.27a, b) can use DNA as sole source of P. There is, of course, the likelihood that both phosphomonoo- and diesters contained in senescent organic residues will be sequestered, along with nitrogenous components, in more complex aromatic and aliphatic macromolecules. Evidence is emerging that some ECM fungi can produce enzymes capable of hydrolysing these 'protected' substrates (see Chapters 15 and 16).

Interest in the ability of ECM fungi to gain access to P sources sequestered in mineral materials has been increased by the recognition that, in some soils, notably those of a podsolic nature, the mycobiont can proliferate in horizons below the superficial organic layers (see Chapter 6). Earlier studies (Stone, 1950; Bowen and Theodorou, 1967) showed that some ECM fungi had the ability to bring P into solution from rock phosphate. There was debate about the mechanisms involved in this release. Acidification of the local environment may be important. In *in vitro* experiments, Lapeyrie *et al.* (1991) concluded that acidification alone could explain release of P from calcium and iron phosphates. When the fungi were grown with ammonium or nitrate as N sources, significant solubilization of P occurred only in the acidic environments produced by ammonium assimilation. More recent studies of the same type (Mahmood *et al.*, 2002) have confirmed the importance of acidification in P release from mineral sources and Rosling *et al.* (2004b) have developed methods enabling quantification of substrate acidification in these systems.

Studies in soils supporting ECM fungal mats (Cromack *et al.*, 1979; Griffiths *et al.*, 1994) implicated a combination of acidification and organic anion production in the processes of P release from these more complex environments. While pH is significantly lower in the mat soils, there are also elevated levels of dissolved organic carbon (DOC) and calcium oxalate. Scanning electron microscopy of minerals in mat soils showed intense chemical weathering which was attributed to oxalate attack in the immediate vicinity of the ECM hyphae (Cromack *et al.*, 1979). While direct evidence for the involvement of the ECM fungi in these processes was not provided, the notion of 'ectomycorrhizal weathering' (rock eating) became established and a considerable literature on the subject has subsequently arisen (van Breemen *et al.*, 2000a, 2000b; Landeweert *et al.*, 2001; Hoffland *et al.*, 2004; Wallander, 2006). There is the suggestion, still unsubstantiated (see Chapter 15), that ECM fungi are responsible for the production of tunnels through mineral particles in podsolic soils (Smits, 2006). While it may inevitably be difficult to distinguish between the P mobilizing activities of ECM fungi from those of the general microflora in natural soil environments, some progress has been made towards evaluation of their roles using defined P minerals in *in vitro*, pot and field experiments.

Some ECM fungi can release P from the potentially important inorganic source of soil P, apatite. Wallander (2000) grew pine seedlings with and without ECM fungi in peat systems to which apatite was supplied as sole additional P source in root-free

compartments. Two of the fungi, *S. variegatus* and an unidentified species, had a significant positive influence on the dissolution of apatite and the seedlings colonized by these fungi produced significantly more biomass than those which were either non-mycorrhizal or poorly colonized by a different isolate of *S. variegatus* (Figure 10.8). A budgeting approach indicated that, after 210 days of exposure to the fungi, ~1% of the apatite had been degraded. There were positive correlations between the amounts of oxalate present in the root-free compartments and P concentrations of their soil solutions, indicating that mobilization of P by the fungi may have been achieved by release of the organic acid.

Considerable differences have been observed in the abilities of ECM fungi to release oxalate (Ahonen-Jonnarth *et al.*, 2000; Casarin *et al.*, 2004) and those fungi like *Rhizophogon roseolus* and *Suillus* spp. with the highest rates of oxalate release are the most effective at freeing P from insoluble inorganic sources (Wallander, 2000; Casarin *et al.*, 2004). Van Schöll *et al.* (2006a) have shown that the production of oxalate and of another low molecular weight organic anion, malonate, by both ECM and non-mycorrhizal seedlings of pine was significantly increased under conditions of P deficiency. More oxalate was produced by seedlings colonized by *Paxillus involutus* than by non-mycorrhizal seedlings, though the latter produced a greater total quantity of organic anions.

Using the mesh-bag method (see Chapter 6), Hagerberg *et al.* (2003) confirmed that growth of ECM fungi in the field could be stimulated by the addition of apatite, but only if the soil was of inherently low P status. Under low P conditions, the quantity of ECM mycelium in apatite-containing bags was 50% greater than in control bags and there was a threefold increase in the number of ECM root tips immediately outside the bags. Such effects could be attributed either to increased allocation

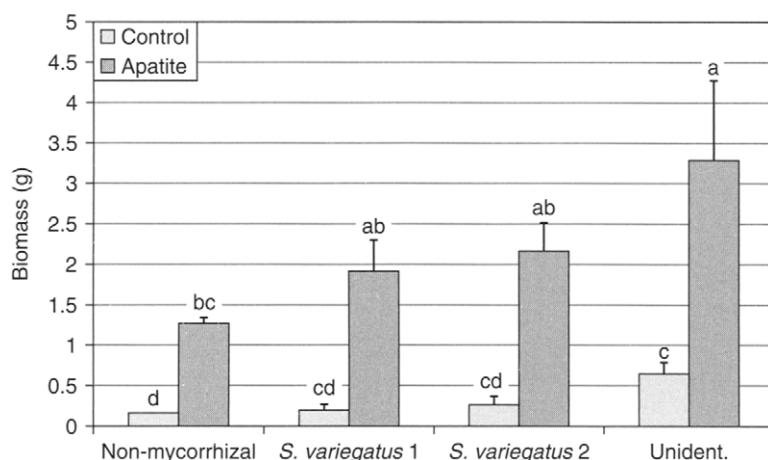


Figure 10.8 Biomass of non-mycorrhizal seedlings and seedlings colonized by one of three different ECM fungi, grown with or without apatite as P source. Bars indicate SE. Different letters indicate statistically different values using two-way ANOVA and LSD to separate the means ($P > 0.5$). Values were log-transformed to equalize the variances of the mean weights of seedlings grown with and without apatite. The P value for effects of EM colonization was 0.001 and for mineral addition $P = 0.000$. The interaction between EM colonization and mineral addition was not significant. From Wallander (2000).

of organic C to each of the fungal species initially present in the soil or to a change in the fungal flora, induced by the greater P availability, in the direction of species which produce more prolific external mycelia. The proliferation of root tips in proximity to the mesh bags suggests that the additional P supply had a strong influence on C allocation by the autotroph. It can be hypothesized that a considerable proportion of the additional C allocated to these resource-enriched areas will be used in the production of oxalate. The ECM-induced dissolution of apatite was 40% greater in the P-deficient soil than in soil adequately supplied with P.

Mobilization, uptake and translocation of potassium

High intracellular concentrations of K are required to maintain activity of enzymes involved in intermediary metabolism, biosynthesis and membrane transport processes. The K⁺ also contributes to the osmotic potential of the cell. Hatch (1937) demonstrated that ECM colonization could enhance the capture of K by plants (see Table 10.1); subsequently, Harley (1978) stressed that the sheath of ectomycorrhizas acts as an important storage organ for many nutrients derived from soil, including K. It was shown that ~67% of the K⁺ absorbed by excised ECM rootlets of *Fagus sylvatica* was retained in the fungal sheath (Edmonds and Harley, unpublished; see Harley and Smith, 1983). Despite these findings, interest in this element has been restricted until recently. Its high coefficient of diffusion in soil should make K⁺ more accessible at absorbing surfaces than P and there has been little to suggest that growth of ECM systems is limited by its availability (Tamm, 1985). However, combinations of high biomass removal and leaching losses arising from anthropogenic soil acidification have led to fears that K could become growth limiting in some environments (Barkman and Sverdrup, 1996; Uebel and Heinsdorf, 1997; Jonsson *et al.*, 2003). Feedbacks from progressively reduced K concentrations in foliage can be expected to lead to reduced availability of the element in soil organic matter. For this reason, there has been renewed interest in the processes whereby K is released from its parent minerals in soils and in determination of the extent to which ECM fungi are involved in its mobilization and transport to the plant.

Among the most important K-containing minerals in soil are the micas phlogopite, biotite and vermiculite, and the silicate mineral, muscovite. It has been shown that, in axenic culture, *Paxillus involutus* can release K from phlogopite and in so doing convert it to vermiculite (Paris *et al.*, 1996). The process was, at least in part, facilitated by exudation of oxalate. Pot experiments comparing the ability of beech either colonized by *Laccaria laccata* or in the non-mycorrhizal condition to obtain K from phlogopite revealed increased K release in ECM treatments (Leyval and Berthelin, 1989) and showed that dual inoculation with *Agrobacterium* further enhanced the effect, apparently largely by acidifying the substrate (Leyval and Berthelin, 1991). In a pot experiment, van Schöll *et al.* (2006b) grew *Pinus sylvestris* either in the non-mycorrhizal condition or with each of three fungal symbionts (*P. involutus*, *Piloderma croceum* and *S. bovinus*) and supplied the plants with muscovite as the sole source of K. Both mineral and non-mineral pools of K were quantified after 27 weeks. Seedlings colonized by *P. involutus* showed almost twofold greater release of K from muscovite than that achieved by the other ECM fungi or by the non-mycorrhizal plants (Figure 10.9). This K mobilization resulted in increased K content of roots

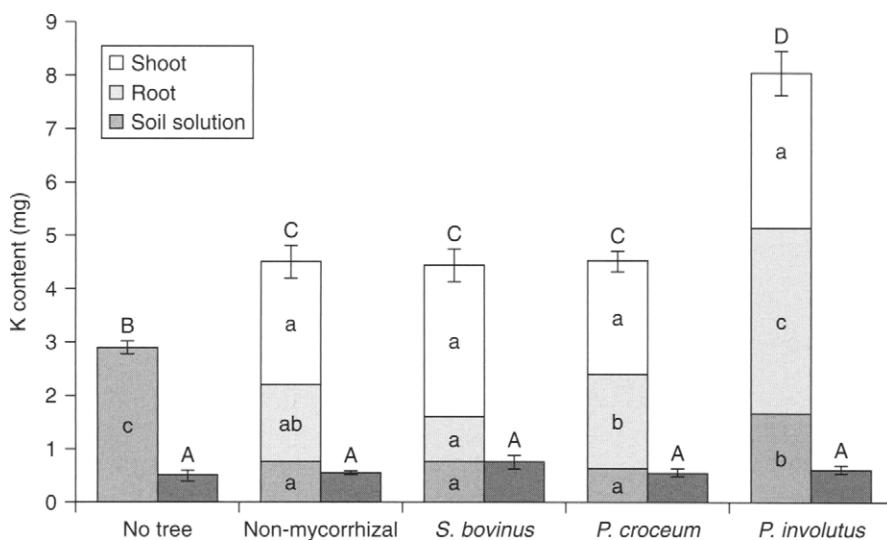


Figure 10.9 Non-mineral-bound K in the soil solution in non-mycorrhizal seedlings of *Pinus sylvestris* and in seedlings mycorrhizal with *Suillus bovinus*, *Piloderma croceum* and *Paxillus involutus* after growth in pots with muscovite as sole source of K. Dark grey bars denote the non-mineral-bound K in pots without muscovite addition. Mean of six replicates. Error bars represent the standard error of the non-mineral-bound K. Bars with the same letter are not significantly different ($P < 0.05$); capital letters above bars denote total amount of non-mineral-bound K in pot. From van Schöll *et al.* (2006b).

and adhering mycelia of the plants. The superior ability of *P. involutus* to release K from muscovite may be attributable to a prolific exudation of oxalate. In a further experiment (van Schöll *et al.*, 2006b), it was shown that, under K-deficient conditions, seedlings ECM with this fungus alone responded by increased production of oxalate. This observation is in line with that of Paris *et al.* (1996).

There is evidence from studies of soils heavily colonized by ECM fungi in the field that K-containing minerals are weathered to a greater extent in the presence of the associated mycelia (Arocena *et al.*, 1999; Arocena and Glowa, 2000). These processes led to a greater concentration of K in soil solution. One of the fungi present in the field, *P. croceum*, which is known to release oxalate in large quantities (Arocena *et al.*, 2001), was shown in laboratory studies to release K from a variety of minerals (Glowa *et al.*, 2003).

There are some discrepancies with regard to observations of the responses of ECM mycelia to the localized addition of K-containing minerals. In field studies, insertion of mesh bags containing biotite did not stimulate mycelial proliferation in the vicinity of the mineral (Hagerberg *et al.*, 2003). This finding is in marked contrast to the situation when the P-containing mineral apatite was added. Conversely, in peat microcosms to which pure potassium feldspar or quartz were added in localized patches, selective proliferation of the mycelia of *Hebeloma crustuliniforme* and *Piloderma fallax* was observed to occur on the feldspar (Rosling *et al.*, 2004b). The differences between the results of the two studies may be attributed to the K status of the surrounding substrates, there being a likelihood that K was more freely available in the forest environment than in the peat of the microcosms. Alternatively, known

differences in the responses of plants to deficiencies of soil P and K may be involved. In non-mycorrhizal birch seedlings, P deficiencies lead to increased allocation of photosynthate to root production, whereas those of K (and Mg) have the opposite effect (Ericsson, 1995), possibly because, in the latter case, there is a tighter coupling between deficiency of the element and C-fixing ability. The same differential effects of the two deficiencies were observed in laboratory studies of ECM pine seedlings (Wallander and Nylund, 1992; Ekblad *et al.*, 1995). Deficiency of K, in contrast to that of P (see above), led to reduced allocation of assimilate to ECM mycelia. Results such as these suggest that the abilities of ECM systems to respond to K deficiency, either by more effective scavenging of available ions or by enhanced weathering of minerals, will be less than they are where P is in limiting supply.

Uptake and translocation of K through ECM hyphae have been demonstrated using Rb⁸⁶ as an isotopic analogue of K (Finlay, 1992) and by mass balance approaches (Jentschke *et al.*, 2001a). Both studies show fluxes of K through the hyphae to be similar in rate to those of P. In the latter study, translocation of K through hyphae of *P. involutus* to ECM spruce plants occurred only when supplementary P had been supplied to the P-deficient system in a root-free compartment. Both this observation and that relating to fluxes of both K and P are consistent with the notion of co-transport of the two elements as K⁺ polyP (see above). Hyphal acquisition of K was estimated by Jentschke *et al.* (2001a) to contribute 6% to total plant uptake of the element. Final calculated and measured K contents of P-fed ECM plants were significantly greater than those in the non-mycorrhizal plants (Table 10.6) confirming that hyphal transport of K can contribute in an important way to whole plant K budgets. Plassard *et al.* (2002) emphasized that K uptake capabilities differed between fungi. Whereas colonization of *Pinus pinaster* by *Rhizophagus roseolus* facilitated a marked increase in uptake of K⁺, no such effects were produced when the plants were colonized by *Hebeloma cylindrosporum*.

Several investigations have explored the extent to which the ECM sheath forms an apoplastic barrier to the entry of solutes, including K to the root tissues. The

Table 10.6 Initial K content, uptake from nutrient solutions and final K contents of mycorrhizal (with *Paxillus involutus*) or non-mycorrhizal Norway spruce (*Picea abies*) seedlings grown in a two-compartment culture system with or without P addition to the hyphal compartment.

Treatment	Initial K content (mg per vessel) week 0	K uptake week 0 – week 11		Final K content (mg per vessel) week 11	
		Plant compartment	Hyphal compartment	Calculated	Measured
Non- mycorrhizal	–P	4.4a	22.7b	–4.3b	22.8b
	+P	3.3a	26.2ab	–5.4b	24.1b
<i>P. involutus</i>	–P	3.2a	27.4ab	–5.6b	25.0b
	+P	4.0a	32.6a	1.9a	38.5a

Data from Jentschke *et al.* (2001a).

apoplastic phase of the fungal mantle of some ECM is impermeable and hence must be a barrier to nutrient transfer between soil and root (Ashford *et al.*, 1988, 1989; Bücking *et al.*, 2002). Ashford *et al.* (1989) showed that entry of the apoplastic marker cellofluor to the roots of *Eucalyptus pilularis* is prevented by the fungal mantle of *P. tinctorius* unless the rootlets were deliberately damaged to remove the outer, unwettable region of the mantle. Bücking *et al.* (2002) investigated the extent to which the ECM mantle formed an apoplastic barrier to the entry of K⁺ into the tissues of the roots. Using the stable isotope ⁴¹K as a tracer they showed that transfer of K⁺ to the root cortex of pine was significantly slower in the presence of mantles formed by *P. tinctorius* and by *S. bovinus* than in non-mycorrhizal roots. The retardation of flow was attributed to hydrophobin production on the mantle surfaces. Time-dependent differences between the fungal species in terms of the patterns of isotope transfer were attributed to distinctive patterns of hyrophobin production on the mantle surface.

The presence of the impermeable layer means that all solutes reach the root cells via the fungal symplast of the mantle, first by translocation in the external mycelium and subsequently by efflux to the interfacial apoplast in the Hartig net region. Conversely, solutes from the root cells effluxing to the apoplast must pass to the fungal symplast and could not 'leak' to the soil via the apoplast of the mantle. The impermeable layers thus offer an opportunity for control of conditions and solute concentrations in the cortical apoplast and Hartig net region, where transport between the ECM symbionts must occur. As Ashford *et al.* (1989) point out, maximum efficiency requires that material must not be allowed to escape from the 'exchange compartment'. The initial work on apoplastic impermeability was carried out with *Pisonia grandis*, in the mycorrhizas of which there is no Hartig net. A similar, but slightly differently organized, exchange compartment could exist where the Hartig net penetrates as far as the outer layer cortical cells; in this case, the inner boundary would be the endodermis, which again provides a block to apoplastic transfer.

The fungal mantle has not been reported to be impermeable in all investigations and the discrepancies may relate to different plant–fungus combinations as well as to different experimental methods. The external mycelium of some species of fungi is also covered with non-wettable material (Unestam, 1991). As yet, it is not clear whether this is relevant to long-distance translocation within the rhizomorphs or whether it might be important in preventing desiccation as has been suggested for the aerial hyphae of some saprophytic fungi, or indeed whether both these attributes are important.

Release, uptake and transport of magnesium

Magnesium (Mg) is a mobile element in soil and, like K, is generally considered to be a non-limiting nutrient in ECM forest soils. However, the anthropogenic factors that are leading to a reduction of K stocks in these environments are also depleting those of Mg. The inability of ECM trees to replenish Mg lost in litter fall has been implicated as a direct cause of decline in European forests suffering from soil acidification (Schulze, 1989).

Van Schöll *et al.* (2006a) examined the ability of ECM fungi to release Mg from the mineral hornblende. In contrast to their observation that *P. involutus* was able to release K from muscovite (see above), they observed no weathering of hornblende by

any of the fungi examined. This may have been because the oxalate-producing capabilities of the fungus, which are stimulated by Mg deficiency (van Schöll *et al.*, 2006b), were inhibited by a rise of pH to over 7.00 caused by addition of the hornblende. Whereas the ability of *P. involutus* to release K from hornblende appears to be small, the fungus has been shown to facilitate access of its ECM associate, *P. sylvestris* to Mg when supplied in root-free compartments (van Schöll, 2006). Hyphae of *P. involutus* proliferate in these compartments in response to the addition of Mg, either as $MgSO_4$ or $Mg_3(PO_4)_2$ (Figure 10.10). The Mg content of the seedlings was also increased (Figure 10.11) irrespective of whether P was supplied as an additional nutrient to the side chamber. This result conflicts with that of Jentschke *et al.* (2001a) who found that hyphal transport of Mg in the same plant-fungus association occurred only under conditions in which supplementary P supplies were provided to the root-free compartment. The difference may be resolved by the fact that, in the study of van Schöll *et al.*, access to Mg was the only factor limiting growth, whereas in that of Jentschke *et al.*, P was limiting and, in the absence of supplementation by this element, hyphal growth and foraging were severely restricted. Jentschke *et al.* (2000, 2001a) used the stable isotope ^{25}Mg to quantify fluxes through hyphae of *P. involutus*. While the values for this element were the lowest of those measured (Table 10.7), fungal translocations were estimated to have contributed approximately 4% of total plant uptake of Mg. Significantly higher concentrations of ^{25}Mg were found in mycorrhizal than in non-mycorrhizal seedlings after the 6 weeks labelling period (Figure 10.12).

It has been shown (Bücking *et al.*, 2002) that, as in the case of K, ECM colonization of pine seedlings significantly reduced the apoplastic transfer of Mg to the root cortex. In roots supporting a fungal mantle, even after an exposure of 72 h to a solution labelled with ^{25}Mg , at least one third of the apoplastic Mg content of the

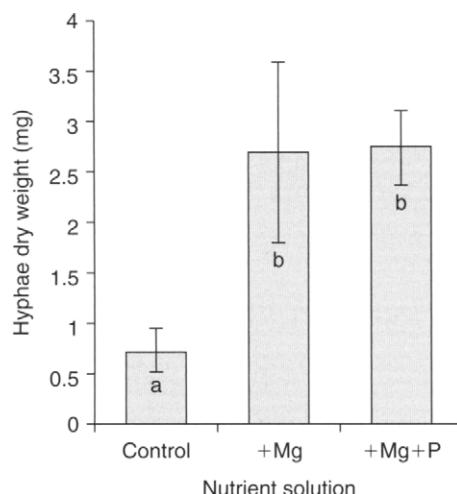


Figure 10.10 Yield of *Paxillus involutus* showing stimulus to hyphal proliferation (expressed as dry weight of mycelium) in root-free compartments to which magnesium (Mg) was added, either alone or with phosphorus (P). Bars with the same letter are not significantly different ($P < 0.05$). Mean of 4 with standard error. From van Schöll (2006).

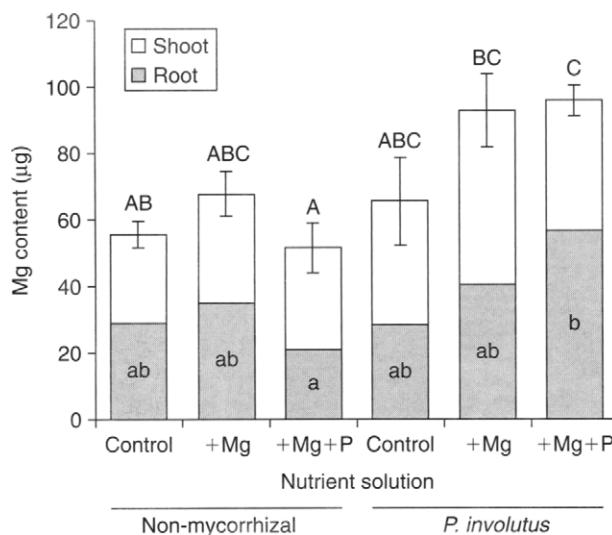


Figure 10.11 Mg content (μg) in shoot and root of *P. sylvestris*, either non-mycorrhizal or colonized by the ectomycorrhizal fungus *P. involutus*. Bars with the same letter are not significantly different ($P < 0.05$); capital letters refer to total Mg content of seedling. Mean of 4–5, standard error for total Mg content. From van Schöll (2006).

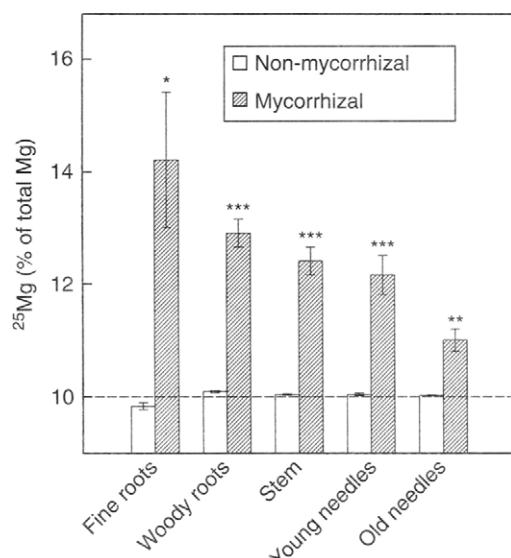


Figure 10.12 Concentration of the ^{25}Mg label (expressed as percentage ^{25}Mg of total Mg) in tissues of non-mycorrhizal and mycorrhizal Norway spruce seedlings after labelling the hyphal compartment in the culture system with ^{25}Mg for 6 weeks. Broken line indicates natural abundance of ^{25}Mg (10.0%). Values are mean values of 4 replicate pots. Small bars indicate standard error. Significance levels for differences between mycorrhizal and non-mycorrhizal seedlings: *, $P \geq 0.01$; **, $P \geq 0.001$; ***, $P \geq 0.0001$. From Jentschke et al. (2000).

Table 10.7 Hyphal translocation of N, P, K and Mg in *Picea abies* seedlings in mycorrhizal association with *Paxillus involutus* during an 11-week experimental period.

Treatment	Hyphal translocation (μm per vessel)			
	Nitrogen	Phosphorus	Potassium	Magnesium
-P	70	nd	nd	nd
+P	660	120	50	7

Data of Jentschke *et al.* (2001a). P and K translocation amounts are from mass balance calculations. N and Mg translocation were determined by stable isotope labelling. Translocation of Mg was corrected for the shorter time period of ^{25}Mg labelling (6 weeks), assuming that the translocation rates were constant over the experiment. nd, not detected.

root cortex had still not exchanged with the externally applied label. In contrast, clear labelling of the apoplastic Mg and complete exchange of the internal and external pools occurred in non-mycorrhizal roots within minutes. These results appear to contrast with those of Kuhn *et al.* (2000) who found that levels of apoplastic ^{25}Mg equilibrated rapidly with those of a bathing medium in ECM roots of spruce. However, sections of spruce roots used in the latter study indicate that, while a Hartig net was well developed, virtually no fungal mantle was present. The absence of the barrier identified by Ashford *et al.* (1989) and Bücking *et al.* (2002) would explain the apparent discrepancy.

Mobilization, uptake and transport of calcium

In contrast to K and Mg, calcium (Ca) is primarily located in cell walls. Its intracellular role as a signalling molecule requires maintenance of extremely low concentrations in the cytosol. Until recently, it has been widely assumed that, as in the cases of K and Mg, supplies of Ca in soils of most ECM ecosystems were sufficient to eliminate the likelihood of deficiency. However, as with those elements, combinations of soil acidification and repeated crop harvesting are now seen to threaten soil Ca stocks in some areas (Yanai *et al.*, 2005). Unfortunately, little is known about the roles of ECM fungi in Ca mobilization and capture. Melin and Nilsson (1955) showed, using the isotope ^{45}Ca , that ECM mycelium could absorb and transport Ca to pine seedlings and mycelial transfer of Ca was confirmed by Jentschke *et al.* (2000). However, Lamhammedi *et al.* (1992) reported reduced levels of Ca in roots of *P. pinaster* plants in ECM association with *P. tinctorius* relative to non-mycorrhizal controls. Similarly van Schöll *et al.* (2005) observed no differences in Ca content of *P. sylvestris* needles when plants were grown in a semi-hydroponic system in the ECM and non-mycorrhizal condition. Bücking and Heyser (2000), using X-ray microanalysis of tissues, found that colonization of *P. sylvestris* by *S. bovinus* and *P. tinctorius* reduced the amounts of Ca detectable in roots, especially in the apoplasts of cortical cells. However, this effect was not observed with *P. involutus* as the mycobiont. An analysis of Ca uptake under different conditions of N supply (Jentschke *et al.*, 2001b) indicated the dynamic nature of the balance between anion and cation uptake.

When N was supplied as ammonium to ECM plants, uptake of Ca (and Mg) was less negatively influenced than in the case of non-mycorrhizal plants. It was concluded that acidification of the whole root compartment of the uncolonized plants led to reduced Ca (and Mg) uptake. In the case of mycorrhizal plants, acidification was localized in the hyphosphere and was less extreme, leading the authors to conclude that the mycelium exerted a buffering effect which ameliorated the negative impacts of ammonium supply on cation uptake.

Effects of ectomycorrhizal colonization on resistance to metal ion toxicity

ECM plants successfully dominate many natural environments where soil acidity and base cation leaching result in exposure to elevated levels of metals. That this exposure has led to some inherent constitutive tolerance to metal pollution in their ECM root systems is shown by the observation that, in many parts of the world, ECM plants successfully colonize mine spoils contaminated with mixtures of metal ions (Meharg and Cairney, 1999). There are reports of enhanced metal tolerance in ECM plants exposed to aluminium (Al) (Cumming and Weinstein, 1990; Hentschel *et al.*, 1993; Schier and McQuattie, 1996; Lux and Cumming, 2001; Ahonen-Jonnarth *et al.*, 2003), cadmium (Cd) (Jentschke *et al.*, 1999), copper (Cu) (van Tichelen *et al.*, 1999), lead (Pb) (Marschner *et al.*, 1996), nickel (Ni) (Jones and Hutchinson, 1986, 1988a, 1988b) and zinc (Zn) (Brown and Wilkins, 1985). However, as emphasized elsewhere (Meharg and Cairney, 1999; Meharg, 2003), in many of these studies tolerance was assessed in terms of growth improvement in the colonized plant and the extent to which the effects were attributable to an enhanced nutrient supply was not resolved.

Evidence that long-term exposure to metals in nature can lead to the selection of constitutively resistant strains of mycobionts was provided by Colpaert and van Assche (1987). Whereas strains of *Suillus bovinus* isolated from Zn contaminated soils could grow in the presence of 1000 µg/g Zn, those from uncontaminated sites produced little or no growth at Zn concentrations above 100 µg/g. Zn-resistant strains of this fungus conferred significantly more tolerance upon plants of *Pinus sylvestris* than did non-resistant strains. In contrast, Denney and Wilkins (1987) could find no evidence that strains of *Paxillus involutus* isolated from Zn-polluted sites had greater ameliorative effects upon birch than strains from unpolluted sites.

The role of fungal sensitivity to a pollutant as a determinant of the potential for amelioration was emphasized by Jones and Hutchinson (1986). Colonization of *Betula papyrifera* seedlings by *Laccaria proxima* or *Lactarius hibbardae* alleviated Ni toxicity at 32 µM Ni, but the effect was lost at 64 µM because, at this concentration, the fungus failed to grow. Similarly, in the case of *Picea* seedlings exposed to Cd, enhanced resistance to the metal at low concentration was lost as amounts increased to the point at which fungal growth was inhibited (Jentschke *et al.*, 1999).

Where metal tolerance is conferred, it may operate at several spatially distinct locations along the mycelium-root-shoot pathway. These have been described by Bellion *et al.* (2006) and involve one or a mixture of routes (Figure 10.13), including extracellular binding on the extramatrical mycelium or fungal mantle by excreted ligands, surface sequestration by binding to the fungal cell wall in the mycelium or mantle, enhancement of efflux from the fungal cell, or by a metallothionein (MT),

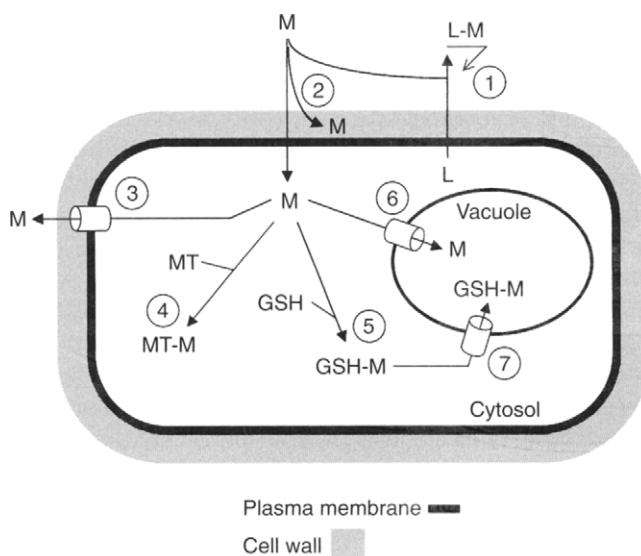


Figure 10.13 Schematic representation of cellular mechanisms potentially involved in metal tolerance by ECM fungi: ① Extracellular chelation by excreted ligands (L); ② cell wall binding; ③ enhanced efflux; ④ intracellular chelation by metallothionein (MT); ⑤ intracellular chelation by glutathione (GSH); ⑥ subcellular compartmentation (vacuole or other internal compartments); ⑦ vacuolar compartmentation of GSH-M complex. M = metal-ion. From Bellion *et al.* (2006).

intracellular chelation by glutathione (GSH), subcellular compartmentation in the cytosol or vacuole, or vacuolar sequestration as GSH complex.

Exclusion of metals by sequestration in the fungal component of the symbiosis contributes to avoidance of toxicity to the plant. The first contact between ECM fungus and a metal is likely to be made by the extramatrical mycelium as it explores the soil. Support for the view that the mycelium provides an important barrier comes from observations that fungi producing the largest quantity of external mycelium provide the greatest resistance to the plant. This has been shown in the cases of Zn (Colpaert and van Assche, 1992) and Cd (Colpaert and van Assche, 1993). Binding of Pb (Jentschke *et al.*, 1991) and Cu (Meharg, 2003) to hyphal surfaces has been demonstrated and there is a possibility that extracellular slime on hyphal walls provides binding sites for metals (Denny and Wilkins, 1987; Tam, 1995). The hyphal surface provides a large number of potential binding sites in the form of free carboxyl, amino, hydroxyl and phosphate groups. A Cu-binding protein exuded into the growth medium by isolates of *L. laccata* and *P. involutus* was considered by Howe *et al.* (1997) to be 'metallothionein-like', but could not be positively identified as such. Subsequently, Courbot *et al.* (2004) have reported a metallothionein-like compound in *P. involutus*. This finding is supported by the presence of a metallothionein sequence, homologous to a known metallothionein from *Agaricus bisporus*, in a cDNA array analysis of *P. involutus* exposed to Cd (Jacob *et al.*, 2004). The expression of this metallothionein gene was examined in the same fungus (Bellion *et al.*, 2006)

and correlations were observed between transcript accumulation and exposure to metals. Metallothioneins can be retained in the cytosol or released to provide extracellular chelation. Courbot *et al.* (2004) showed that, in contrast to metallothioneins, there was a complete lack of phytochelatins in *P. involutus*.

The possibility that exudation of organic anions can lead to chelation of potentially toxic ions has been more extensively investigated. Bellion *et al.* (2006) report experiments showing that exposure to oxalic acid reduced Cd uptake in *P. involutus* by 85%. Of seven organic anions examined, oxalate provided by far the most effective metal binding activity. Ahonen-Jonnarth *et al.* (2000) showed that pine seedlings mycorrhizal with *Suillus variegatus* and *Rhizophagus roseolus* responded to Al exposure by strongly increasing release of the effective Al-chelator oxalate. Subsequently, it has been reported (van Schöll *et al.*, 2006a) that, while oxalate production was greatest in seedlings suffering Mg and P deficiency, no exudation occurred in the absence of Al ions. Large differences between fungal symbionts were observed in their ability to release oxalate. Indeed, differences in Al-induced organic anion exudation among seedlings colonized by different ECM fungi were as big as or bigger than those between non-mycorrhizal and ECM seedlings. Much more needs to be learned about the relative importance of the different processes involved in the exclusion of toxic metals and we still know little about the duration over which sequestered metals can be immobilized in the hyphosphere.

Depending upon the extent of its hydrophobicity, some extracellular localization of metals can also be expected to occur on, or in, the ECM mantle. Using X-ray microanalysis of cryosections, Frey *et al.* (2000) showed that two distinct mechanisms were involved in the binding of Zn and Cd ions in ECM roots. In the case of Cd, extracellular complexation occurred primarily in the Hartig net and in the cell walls of root cortical cells, indicating that transfer from fungus to plant occurred readily and that the pathway was primarily apoplastic. In contrast, Zn accumulated mainly in the cell walls and cytoplasm of the mantle hyphae, there being less transfer to the plant. A wide range of potentially toxic elements were shown to accumulate in the fungal mantles of *Pinus* roots growing on contaminated soil (Turnau *et al.*, 2002).

There have been relatively few analyses of the response of mycobionts to accumulation of metals. Metals absorbed by the external hyphae, mantle or Hartig net represent a threat to cellular metabolism. Oxidative stress is one such effect of entry and the activities of a number of enzymes released in response to this threat have been investigated after exposure of *P. involutus* mycelium to Cd ions (Ott *et al.*, 2002). The work confirmed that superoxide dismutase (SOD), catalase, glutathione reductase and glutathione peroxidase were active in the mycelium of the fungus. Ott *et al.* (2002) concluded that *P. involutus* is able to detoxify high concentrations of Cd by a strong induction of glutathione synthesis, accompanied by a rapid sulphur-dependent transport of Cd into the vacuole. Glutathione reductase activity was increased by exposure to low levels of Cd. A SOD gene was shown to be upregulated and to be under post-translational control following exposure of *P. involutus* to Cd ions (Jacob *et al.*, 2001). Using a desorption method with ^{109}Cd , Blaudez *et al.* (2000) showed that 20% of the Cd added to cultures of *P. involutus* appeared in the hyphal cytosol, while 30% was transported to the vacuole. Vacuolar accumulation of Cd was suggested to be an essential detoxification mechanism in this fungus. Enhancement of efflux or downregulation of genes involved in uptake of metals may be effective in providing avoidance of toxicity in some cases (Adriaensen *et al.*, 2005).

Searches of GenBank for sequences from a range of ECM fungi have revealed the presence of numerous expressed sequence tags (ESTs) or open reading frames that encode for proteins known in the yeast *Saccharomyces cerevisiae* to be involved in metal tolerance pathways (Table 10.8) (Bellion *et al.*, 2006). The demonstration that particular gene products are involved in increasing metal tolerance remains to be achieved in almost all cases and transformation systems enabling overexpression or disruption of target genes in ECM are urgently required in order to test their roles in contaminated soils.

Effects of ECM colonization on plant water relations

There has long been an interest in the possibility that ECM colonization might improve the water economy of trees and there are some reports that the symbioses can provide a measure of drought resistance at intermediate levels of water stress (Cromer, 1935; Zerova, 1955; Goss, 1960). However, as few of the early observations were accompanied by measurements of tissue water balance, it remains a possibility that such benefits could be attributable to nutritional rather than hydrological effects.

Advances were achieved by studies in which tissue water potentials were compared in ECM and non-mycorrhizal plants exposed to the same controlled drought treatments. Dixon *et al.* (1983) showed that the pre-dawn water potentials of container-grown *Quercus velutina* exposed to modest drought were higher in plants ECM with *P. tinctorius*, than in those lacking colonization. Similar effects of ECM colonization by this fungus were observed with *Pinus virginiana* (Walker *et al.*, 1982), *P. taeda* (Walker *et al.*, 1989) and *P. halapensis* (Morte *et al.*, 2001). Even in cases such as these involving monitoring of tissue water balance, it is not possible to discriminate between direct impacts of ECM formation on the uptake and transport of water and indirect effects arising from nutritional differences between colonized and uncolonized plants.

Comparative analyses of the water transport pathways of ECM and non-mycorrhizal plants provide a better view of the relative hydraulic conductances. In the case of *Ulmus americana*, ECM colonization leads to significant increases of apoplastic water transport and root hydraulic conductivity, these effects being maintained across a temperature range (Figure 10.14) (Muhsin and Zwiazek, 2002). On the basis that transmembrane water transport is mediated by aquaporin proteins (Maurel and Chrispeels, 2001), these authors hypothesized that application of a mercuric protein inhibitor would enable the relative importance of transmembrane and apoplastic transport to be determined. Since the application of the inhibitor had a lower impact on conductance in ECM than non-mycorrhizal roots, it was concluded that reduced conductivity at lower temperatures in the symbiotic condition were mediated more by changed water viscosity than by impacts on the membrane channels themselves. Nonetheless, a combination of apoplastic and membrane-based processes were considered to contribute to the increase in hydraulic conductance seen in the ECM plants.

The importance of aquaporins as determinants of water flow in ECM plants has been highlighted by elegant studies involving a combination of molecular and physiological analyses using *Populus* hybrids and the ECM fungus *Amanita muscaria* (Marjanovic *et al.*, 2005a, 2005b). It was first shown that, as in the case of *Ulmus* (see above), ECM roots had significantly higher hydraulic conductances, in this case

Table 10.8 Putative proteins from ectomycorrhizal fungi similar to proteins belonging to yeast metal tolerance pathways

Mechanism	Pathway	Function	Organism	GenBank accession no
Transcription factors	YAPI-like	Regulation of genes involved in oxidative stress tolerance and metal resistance	<i>Tuber borchii</i>	CN488390
	ZAPI-like	Regulation of zinc transporters	<i>Paxillus involutus</i>	CN072154
Transport systems involved in metal tolerance and homeostasis	Metal efflux into organelles	Cation diffusion facilitator	<i>Hebeloma cylindrosporum</i>	CK993155
		Cd-conjugate ABC transporter	<i>Hebeloma cylindrosporum</i> <i>Pisolithus microcarpus</i>	CK995083, CK992826 CB10722
	Metal influx	Metal-transporting ATPase	<i>Hebeloma cylindrosporum</i> <i>Tuber borchii</i>	CK992318, CK994170 AF487323
		Manganese transporter	<i>Hebeloma cylindrosporum</i>	CK995213, CK992324, CK995203
		Copper transporter	<i>Tuber borchii</i>	CN487781
		Iron transporter		
Intracellular metal binding	Metal delivery to other proteins	Metallochaperone	<i>Paxillus involutus</i>	AAT91247, AAT31333, AAT91334 AAT91335, AAT91336, CD273262
				CD273746, CD273829, CD275306 CD274894
	Cu and Cd binding Glutathione synthesis		<i>Hebeloma cylindrosporum</i>	BU964154
		Metallothionein γ -glutamylcysteine synthetase	<i>Paxillus involutus</i> <i>Hebeloma cylindrosporum</i>	AAS19463 CK995328
		Glutathione synthetase	<i>Paxillus involutus</i> <i>Paxillus involutus</i>	CD273087 BG141319

(Continued)

Table 10.8 (Continued)

Mechanism	Pathway	Function	Organism	GenBank accession no
Protection against metal-induced oxidative stress	Regulation of cell redox homeostasis	Thioredoxin	<i>Paxillus involutus</i>	AAS19462, CD275083, CD275423, CD276018
			<i>Hebeloma cylindrosporum</i>	CK995145, CK995656
		Glutaredoxin	<i>Tuber borchii</i>	BM26656, CN487764, CN48812
			<i>Pisolithus microcarpus</i>	CB011224, BF942541
			<i>Laccaria bicolor</i>	CB012066
	Removal of reactive-oxygen species	Catalase	<i>Tuber borchii</i>	BM266155
			<i>Pisolithus microcarpus</i>	BF942586
		Superoxide dismutase	<i>Laccaria bicolor</i>	CB10230, CB010243
			<i>Laccaria bicolor</i>	CB10617
			<i>Tuber borchii</i>	BM266201
Data from Bellion et al. (2006). Selected protein sequences identified in <i>Saccharomyces cerevisiae</i> being involved in metal tolerance pathway were used to search for expression sequence tags or open reading frames from ectomycorrhizal fungi encoding putative proteins similar to them. Searches were made by TBLASTn or BLASTp in the NCBI database ($P = <0.05$).				

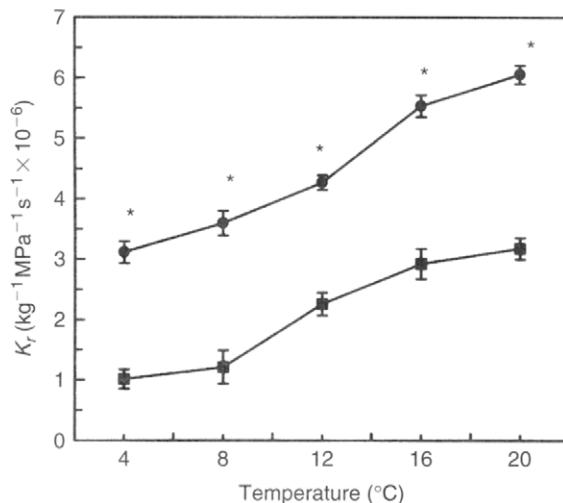


Figure 10.14 Root hydraulic conductance (K_r) of mycorrhizal (●) and non-mycorrhizal (■) seedlings *Ulmus americana* exposed to decreasing temperatures. Mean ($n = 8$) \pm SE are shown.* indicate statistically significant difference ($P = 0.05$) between mycorrhizal and non-mycorrhizal roots. From Muhsin and Zwiasek (2002).

greater by up to 40% (Figure 10.15), than their non-mycorrhizal counterparts. Since the root systems of ECM plants had a surface area lower by 17% than that of the non-mycorrhizal plants, the real difference in hydraulic conductance between the two systems was larger, equivalent to an increase of 57% in water transport capacity (Marjanovic *et al.*, 2005a). Seven genes coding for aquaporins were isolated from a poplar ectomycorrhizal cDNA library and it was shown that four of them were preferentially expressed in roots prior to ECM formation. After colonization by *A. muscaria*, three showed an increased transcript accumulation including two (*PttPIP1.1* and *PttPIP2.5*), which are the most commonly expressed aquaporins in roots (Figure 10.16). When expressed in *Xenopus* eggs, these genes were confirmed to have the capability to transport water. The high expression, in particular of *PttPIP2.5*, associated with ECM colonization, suggests that these aquaporins play a key role in facilitating the increased water transport capacity seen in the ECM plants. It was subsequently shown (Marjanovic *et al.*, 2005b) that the expression of two aquaporin genes was more pronounced under conditions of drought in ECM poplar plants, indicating that the symbiosis may improve the capacity to transport water during periods of reduced supply.

Indirect evidence in support of the view that the extraradical mycelia might serve as conduits for water supply to plants was obtained by cutting the rhizomorphs of *Suillus bovinus* which were growing from a colonized plant into moist soil (Boyd *et al.*, 1986). Severance of the connections led to an almost instantaneous decline in plant transpiration. The importance of the external mycelial phase for water absorption was further emphasized by Lamhamadi *et al.* (1992) who examined the ability of a number of genetically distinct dikaryons of *P. tinctorius* to influence xylem water potential of *P. pinaster* growing under moderate drought. Significant correlations were found between plant water potential, total root system resistance

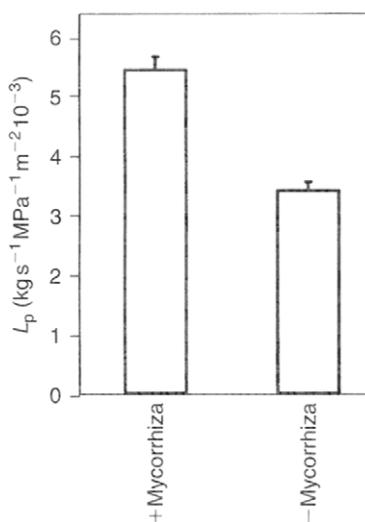


Figure 10.15 Measurements of the root hydraulic conductivity of mycorrhizal and non-mycorrhizal poplar root systems. Root hydraulic conductance was measured with a high-pressure flow meter in the excised undisturbed root systems of mycorrhizal and non-mycorrhizal poplar plants and used (together with the size of the root systems) to calculate the root hydraulic conductivity (L_p). From Marjanovic *et al.* (2005a).

and both the extension growth and rhizomorph diameters of the different fungal strains (Figure 10.17). Those genotypes that produced the most extensive systems of thick rhizomorphs enabled their plant partners to sustain the highest xylem water potential when soil water potential was low.

It is well known that soil water potentials can be reduced, particularly in surface horizons containing a high proportion of the ECM roots and mycelia, as water is extracted during the day by the demands of the transpiration flux. Predictably, this drying can have adverse effects upon the activities of the ECM symbiosis (Nilsen *et al.*, 1998; Swaty *et al.*, 1998) as well as on nutrient availability. However, after stomatal closure at dusk, the accumulated tissue water deficits are sufficient under some circumstances to enable roots to absorb and raise water from deep soil layers in a process termed 'hydraulic lift' (Caldwell and Richards, 1989). It has been hypothesized (Caldwell *et al.*, 1998; Horton and Hart, 1998) that recharging of the water resources of the surface layers in this way could facilitate retention of mycorrhizal activity in what would otherwise be dry soil. In a test of this hypothesis, Querejeta *et al.* (2003) constructed compartmented microcosms enabling water with coloured tracers to be supplied to tap roots of droughted ECM live oaks (*Quercus agrifolia*) at dusk or dawn. Microscopic analysis of fine roots and their associated AM and ECM symbionts in the uppermost compartment at dawn, following feeding of tracer to the basal units at dusk, revealed extensive labelling of both root tissues and external mycelium in the surface soil. After feeding at dawn, no such upward movement was detected. Because the tracers used were membrane-impermeable, it was concluded that the pathway for water transport must be apoplastic, rather than by a process of leakage from plant tissues that would require reabsorption across fungal membranes.

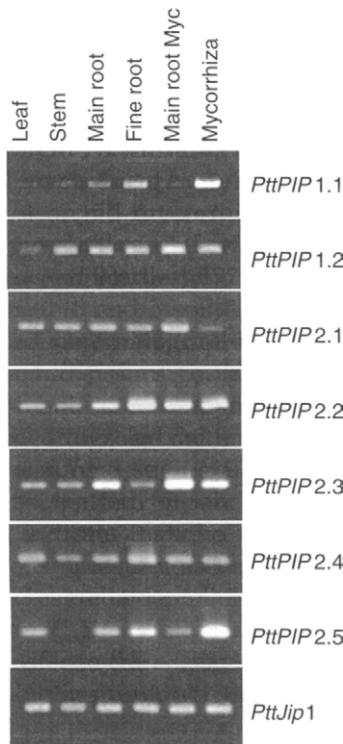


Figure 10.16 Expression profiles of *Populus tremula* × *tremuloides* plasma membrane proteins (*PttPIPs*) (i.e. aquaporins) in poplar organs and ectomycorrhizas. Total RNA was isolated from leaves, stems and roots (separated into fine and main roots) of non-mycorrhizal and mycorrhizal plants. Upregulation of *PttPIP1.1* and *PttPIP2.5* is observed in the mycorrhizal roots. From Marjanovic *et al.* (2005a).

Redistribution of dye through rhizomorphs of ECM fungi over distances of several centimetres from the root was observed.

Clearly, the potential implications of these observations for the functioning of both mycorrhizas and the wider ecosystem are large. It can be envisaged that hydraulic lift would be particularly advantageous in systems subjected to surface drying during the growing season. There is now a need to explore the extent to which this phenomenon occurs in nature and to quantify its effects in terms both of water supply to the symbionts and facilitation of nutrient mobilizing potentials.

Water shortages in soil can be expected to have influences upon the fungal community as well as upon the plants. There is ample evidence that drought can cause major changes both in the amount of ECM colonization present (Nilsen *et al.*, 1998; Bell and Adams, 2004), in the structure of the fungal communities (Swaty *et al.*, 1998, 2004; Shi *et al.*, 2002) and their physiological activities (Jany *et al.*, 2003; Bell and Adams, 2004). These impacts are likely to have lasting effects, especially if they arise as a result of loss of species that function most effectively under normal conditions of moisture supply. In those circumstances where hydraulic lift occurs, the process may provide some buffering of the fungal community against the effects

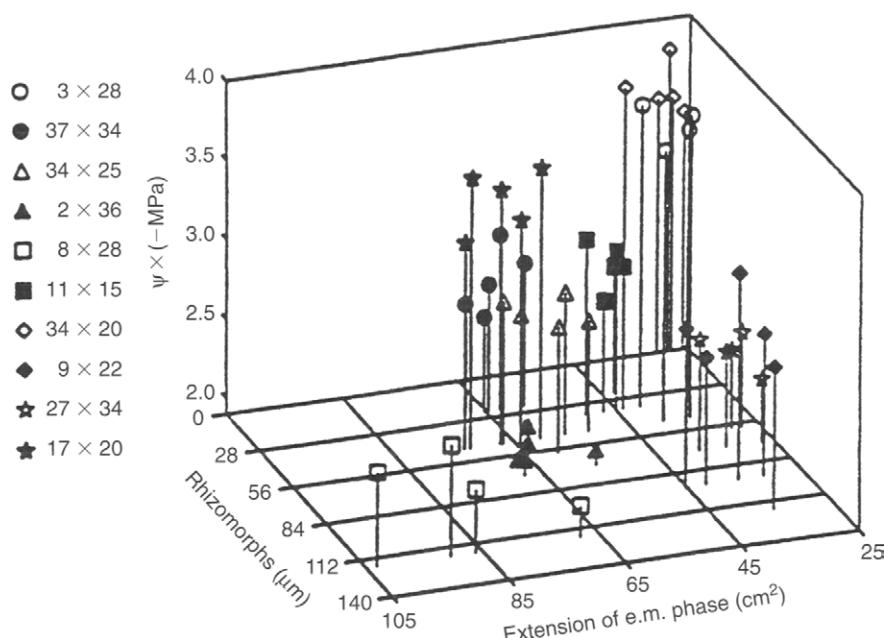


Figure 10.17 Effects of the diameter of rhizomorphs (μm) and of extension rates of the extraradical mycelial systems (cm^2) of a range of dikaryotic isolates (designated by number on left of figure) of *Pisolithus tinctorius*, upon the xylem water potential (ψ_{xylem}) of *Pinus pinaster* seedlings. From Lamhamadi et al. (1992), with permission.

of modest drought, but is unlikely to prevent the lethal impacts of prolonged or severe drought. Further, if hydraulic lift increases soil moisture content, effects both on activities of soil saprotrophs and on nutrient availability may also contribute to beneficial effects.

The differential ability of ECM fungi to withstand reductions of water potential when grown in pure culture has been adequately established (Mexal and Reid, 1973; Theodorou, 1978). Coleman et al. (1989) observed that all ECM fungi examined showed reduced growth as water potential decreased. Some, like *Laccaria laccata*, failing to grow below -1 MPa , whereas others, such as *Suillus granulatus* and *Cenococcum geophilum*, were able to grow at -3 MPa . These patterns of relative drought sensitivity are reflected to some extent in nature where drought is reported to promote increases of colonization by fungi like *C. geophilum* (Mikola, 1948). Pigott (1982) reported that colonization of *Tilia cordata* by this fungus enabled roots to survive in soils to water potentials as low as -5.5 MPa . However, in the absence of experimental analyses of the effects of *C. geophilum* on plant water relations, we are still unable to establish whether the increased presence of this fungus under conditions of drought makes any contribution to improving plant performance.

Conclusions

Early work emphasized the role of the fungal sheath in the processes of absorption, storage and transfer of P and laid the basis for our understanding of the physiology

of nutrient transfer between fungus and plant in the ECM symbioses. Most of these studies were carried out using excised roots, but emphasis has moved more recently towards consideration of intact systems in which the effectiveness of different fungi in capture, transport and transfer of P to the plant has been examined in soil. The relationship between P acquisition and growth is now much more clearly understood and a number of studies have provided estimates of P inflow in intact ECM systems. These are of the same order of magnitude as those seen in AM systems. The role of the extraradical mycelium in exploring the soil and facilitating the mobilization of P from complex sources, both inorganic and organic, has been emphasized. The success of combined laboratory and field-based studies in elucidating the mechanisms and significance of increased P capture is clear. Both production of phosphatases, which release P from organic sources, and production of protons and organic anions, which can accelerate processes of chemical weathering, appear to be important in soils that have major mineral components. There is now a need to characterize more precisely the chemical nature of the main sources of P used by ECM plants in nature and to investigate the relative effectiveness of different species and races of fungal symbionts in providing access to them. Considerations of P uptake cannot be made in isolation. In many natural environments, P and N occur together in organic substrates and their release requires a complex suite of enzyme activities, some of them involving prior breakdown of polymeric carbon sources. We know little of the relative abilities of ECM and non-mycorrhizal microbial communities to achieve this breakdown, or of any competitive interactions that may take place between them.

We are beginning to recognize that, in addition to P and N limitation in forest ecosystems, other key elements (notably K, Mg and Ca), the availability of which has hitherto been largely taken for granted, may soon be in short supply. Clearly, we know a great deal less about the role of ECM fungi in the release, absorption and transfer of these elements and much more work needs to be done. There is much to suggest that through their abilities, both locally to secrete low molecular weight organic anions and more widely to release protons, these organisms may be critically involved in important biogeochemical weathering processes, as well as in the transfer of released cations to the plant. However, we still know too little about the relative contributions of ECM and saprotrophic communities to the overall mineral weathering budget to draw firm conclusions with regard to the role of the symbiotic community in the biogeochemistry of ectomycorrhizal environments dominated by ECM plants. Likewise it is difficult, on the basis of the small number of experiments carried out to date, to determine whether and under what circumstances ECM colonization can improve plant access to base cations. It also emerges not only that ECM colonization can significantly change both the processes of transfer to, and the balance of cations within, the tissues of the plants, but also that the mycobionts differ greatly among themselves in their propensities to influence cation supply to the root.

Recent work has extended knowledge of ECM symbioses beyond plant nutrition, to encompass aspects of responses to stresses. It is clear that ECM fungi have the ability to confer tolerance to both heavy metals and drought. In both cases, part of the beneficial effects may stem from improved nutrition, but it is also clear that direct effects conferred by activities of the fungal partners are implicated and that there is considerable diversity in responses, dependent on the identity of symbiotic partners. The significance both of enhanced tolerance and of symbiont-dependent effects in field situations requires ongoing investigation.