

Metal toxicity and ectomycorrhizas

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Metal toxicity (Al and heavy metals) is a major constraint affecting root growth in a number of natural or managed ecosystems. Fine roots of the majority of plant species are associated with mycorrhizal fungi, which may modify the sensitivity of roots to metal stress. In this review, we summarise the available evidence demonstrating beneficial effects of ectomycorrhizas in alleviation of metal toxicity in forest tree seedlings. We identify experimental shortcomings of past research (e.g. the use of shoot metal concentrations as a measure of metal uptake, use of microanalytical techniques biased by element redistribution) that may confound major

conclusions drawn from these experiments. Although there is no doubt that in many cases ectomycorrhizal fungi indeed ameliorate metal stress in their host plants, the mechanism(s) involved remain(s) unclear. The role of metal sorption on fungal tissues thought to reduce metal exposure of the host plant is critically reviewed. As direct evidence (both under artificial and soil conditions) supporting a unique role of fungal immobilisation of metals is lacking so far, there is an urgent need to also test alternative tolerance mechanisms such as the release of metal chelating substances, or nutritional and hormonal effects mediated by mycorrhizal fungi.

Introduction

In temperate forests, fine roots of trees are almost entirely mycorrhizal. Anthropogenic impacts on forest ecosystems such as acid and nitrogen deposition, rises in atmospheric CO₂, contamination with metals and/or organic substances, can strongly influence mycorrhizal community structure. As a result a loss of mycorrhizal diversity can occur (e.g. Wainwright and Gadd 1997). To understand the consequences of these changes on root growth and tree performance, and in order to develop strategies for the restoration of affected forest ecosystems, we need to gain a deeper understanding of the function of mycorrhizas in ecosystems and the mechanisms by which they promote these functions.

Metal toxicity (Al and heavy metals) has been hypothesised to play a crucial role in 'new-type forest decline' in Central Europe and North America. However, the presence of mycorrhizas may modify the sensitivity of plants to metals. Early work on ectomycorrhizas showed that inoculation of conifer roots with *Pisolithus tinctorius* greatly improved seedling survival and growth on mine spoils containing high levels of metals and extreme acidity (e.g. Marx and Artman 1979). As tree seedlings growing on mine spoils often suffer from a range of stresses, including drought or mineral nutrient deficiency, these early results

did not unequivocally demonstrate a reduction in metal toxicity by fungal colonisation. However, this work has provoked much interest in the role of ectomycorrhizas in alleviation of metal toxicity, and has led to a great number of publications in this field. This review aims to summarise the available information on amelioration of metal toxicity by ectomycorrhizal associations, and to highlight the potential mechanisms involved. Although effects of metals on the fungal side of the mycorrhizal symbiosis, including the colonisation potential under metal stress, are very important for understanding the impact of metals on mycorrhizal plants, this will not be considered in detail here. For this, the reader is referred to reviews by Hartley et al. (1997), Leyval et al. (1997) and Rapp and Jentschke (1994).

Experimental approach

In physiological studies on the response of mycorrhizas to environmental stress, a number of culture techniques have been employed. These fall into two major groups: those which use a solid substrate for rooting and those that do not. The latter comprises of classical solution culture tech-

niques (e.g. used by Kamminga-vanWijk et al. 1992) or modifications thereof (e.g. Göransson and Eldhuset 1991). For studies on metal toxicity, hydroponic culture has a great advantage, as it is not biased by solid phase-solution interactions. However, this technique is not frequently used as ectomycorrhizal development can be significantly reduced by prolonged submergence even with an adequate supply of oxygen (Stenström 1991). In the majority of investigations, artificial solid substrates (vermiculite, peat, perlite or sand) have been used. On these substrates, metal ions may be adsorbed by ion exchange or other processes. In addition, ions may be released from the substrate, examples being, the release of organic compounds from peat resulting in complexation of metals or K dissolution from vermiculite, and Al dissolution from perlite under acidic conditions (Metzler and Oberwinkler 1987). The released cations may then reduce the toxicity of the metal investigated as a result of cation competition for binding sites on plant surfaces (Kinraide 1998). In addition, high pH or high nutrient concentrations (especially P and S) may interfere with Al or heavy metals by altering metal speciation or precipitation of metal salts. Hence, metal effects in systems containing solid substrates and/or high nutrient concentrations may be altered in a complex way.

Although experiments under artificial conditions are necessary to unravel the mechanisms involved in mycorrhizal effects on metal tolerance, they cannot tell whether the proposed mechanisms act under natural conditions. For this reason, experiments with plants growing in natural soil are necessary. However, to date, only a few investigations (e.g. Dixon 1988) have used plants grown in soils containing elevated levels of metals.

As a measure of the 'amelioration capacity' of mycorrhizas, researchers have used an array of parameters characterising plant growth, nutrient uptake, photosynthesis, transpiration, biochemical compounds and processes, metal uptake or metal distribution on various scales, which also included microanalytical techniques on the tissue, cellular and subcellular level (see Hartley et al. 1997). Researchers should critically evaluate whether the chosen parameter is closely linked and relevant to the expression of metal toxicity to avoid misinterpretation of results.

Mycorrhizas and metal sensitivity of tree seedlings

Seedling growth

Although a number of publications have claimed to show alleviation of metal toxicity by ectomycorrhizas, only a few papers have presented direct evidence for such an effect. In these studies, ectomycorrhizal fungi have been demonstrated to alleviate growth depressions of tree seedlings due to the toxic effects of Al (Cumming and Weinstein 1990a,b, Hentschel et al. 1993, Schier and McQuattie 1995, 1996), Ni (Jones and Hutchinson 1986, 1988a), Zn (Brown and Wilkins 1985) and Cd (Jentschke et al. 1999). For other important heavy metals, for example, Hg and Pb, direct evidence of amelioration by ectomycorrhizal fungi is still lacking. This is predominantly due to the lack of any

experimental data (e.g. Hg) or the lack of a significant toxic effect on the non-mycorrhizal seedlings (Pb; Jentschke et al. 1991a, Marschner et al. 1996).

Nutrient uptake

In addition to reduced root growth, reduced uptake of Ca and Mg is a common feature accompanying Al phytotoxicity (Marschner 1991). In general, Al seems to affect the uptake of Ca and Mg similarly in mycorrhizal as in non-mycorrhizal seedlings (e.g. Hentschel et al. 1993, Schier and McQuattie 1995, 1996). Long-term exposure of mycorrhizal tree seedlings may therefore lead to the development of Mg deficiency symptoms and reduced photosynthesis (Hentschel et al. 1993). Since the inhibition of photosynthetic CO₂ uptake affects root and shoot growth of the mycorrhizal seedlings, it may be expected that the ameliorating effect of the mycorrhiza may decrease or even disappear after prolonged exposure to Al. Aluminium may also interfere with P uptake (Marschner 1991). Cumming and Weinstein (1990a) found that exposure to Al reduced P uptake in non-mycorrhizal but not in mycorrhizal *Pinus rigida* seedlings. The significance of this will be discussed in 'Mechanisms of amelioration'.

Recently, VanTichelen et al. (1999) have shown in short-term experiments that *Thelephora terrestris* and *Paxillus involutus* offset Cu-induced inhibition of NH₄⁺ uptake in pine seedlings, although growth was not affected. These results, however, need confirmation, as the method used to measure nutrient uptake did not differentiate between NH₄⁺ uptake by the host plant, the mycobiont and the associated rhizosphere microflora.

Factors affecting amelioration

From the very few investigations showing clear toxic effects of metals on the host plant, and in which more than one fungus was used (Jones and Hutchinson 1986, 1988a,b, Denny and Wilkins 1987a, Jentschke et al. 1999, VanTichelen et al. 1999), it is clear that, in general, the ameliorating effect strongly depends on the fungal species. This suggests that some fungi are effective ameliorators and others are not. The reasons for this variation are poorly understood (Hartley et al. 1997). Although it may be expected that ecotypes of mycorrhizal fungi adapted to high metal environments confer a higher metal tolerance to the host plant, there is no direct evidence supporting this hypothesis. Papers attempting to show such an effect (Colpaert and van Assche 1992, 1993) were inconclusive as amelioration in terms of improved growth was not shown. Denny and Wilkins (1987a) showed that different ecotypes of *Paxillus involutus* differentially affected the growth of birch clones at high Zn. Unfortunately, comparative non-mycorrhizal controls were not included in this experiment. However, no relationship was found between the origin of the strain (isolated from high or low Zn status soils) and its effect on the growth of the birch plants. Bücking and Heyser (1994) found that a strain of *Suillus bovinus*, after being adapted to high Zn, reduced Zn translocation to the shoots of *Pinus*

sylvestris seedlings. As this strain also reduced root growth after adaptation to high Zn, it is, however, difficult to draw firm conclusions from this experiment. In addition, as will be outlined below, shoot metal contents are problematic as a measure of the degree of amelioration.

Not only does the fungal species or genotype determine the efficiency of the ameliorative effect, but it is also influenced by abiotic factors, most important of which is the metal concentration of the rooting substrate. Jones and Hutchinson (1986) found that colonisation by *Laccaria proxima* or *Lactarius hibbardae* alleviated Ni toxicity in *Betula papyrifera* seedlings at 32 μM Ni but not at 64 μM . Similar results were found in mycorrhizal Norway spruce seedlings for Cd (Jentschke et al. 1999). A fungus can only increase the tolerance of its host, if fungal tolerance exceeds that of the host plant. In the experiment with Cd, the fungus may have been affected by the high Cd treatment (5 μM), thus losing its ability to alleviate Cd toxicity. However, indirect evidence suggested that the fungus was still viable at 5 μM Cd, indicating that the mechanism of amelioration does operate up to a certain threshold of metal exposure only.

Effective amelioration may also depend on the specific metal. Jones and Hutchinson (1986) demonstrated that alleviation of Ni toxicity does not necessarily imply tolerance to other metals (e.g. Cu). None of the fungal strains, which successfully reduced the sensitivity of the host to Ni, did this with respect to Cu.

Rhizosphere pH is an important factor in metal toxicity (Marschner 1991), and may influence the efficiency of mycorrhizal amelioration. Rhizosphere pH is highly dependent on N nutrition. The effects of N source on mycorrhizal amelioration of Al toxicity were investigated by Cumming's group (e.g. Cumming and Weinstein 1990b). While Al inhibited root growth of non-mycorrhizal *Pinus rigida* seedlings supplied with NO_3^- , there was no such effect when NH_4^+ was present, probably as a result of the high SO_4^{2-} concentrations used in the nutrient solutions causing a significant complexation of Al. Hence, the amelioration efficiency at different N sources could not be compared conclusively.

Mechanisms of amelioration

Metal tolerance of higher plants may be due to a range of potential processes (e.g. Ernst et al. 1992). These may include (1) a reduction of metal exposure by excretion of chelating substances, (2) extracellular sequestration (e.g. by mucilage, pH gradients in the rhizosphere), (3) modified uptake systems at the plasmalemma, or (4) intracellular detoxification. The significance of these processes may vary as a function of the metal involved, its concentration, and the location of the primary lesion caused by the metal.

Mycorrhizal fungi may alter metal sensitivity of their hosts, theoretically, by any of the mechanisms outlined above, by either directly affecting metal availability and speciation (Fig. 1) or indirectly modifying plant physiological processes, for example, by phytohormone action. Given the broad spectrum of fungi forming ectomycorrhizal associations, it is likely that different fungi may affect metal

sensitivity of their hosts by different mechanisms. Despite a great number of potential mechanisms, research, however, has almost exclusively focussed on metal immobilisation within fungal structures. Hence, data are too scarce to allow critical evaluation of alternative mechanisms.

Metal mobility in the fungal apoplast: filtering of toxic metals in the hyphal sheath or Hartig net by adsorption (exclusion mechanism 1)

Fungi can effectively bind metals to cell walls (Gadd 1993) or extracellular polysaccharides (see later). In addition, intracellular uptake and accumulation of certain metals in vacuoles may be significant, although this has not been clearly documented for filamentous fungi (Ross 1993), and will not be considered in detail here. Binding of toxic metals to cell walls has been suggested as a tolerance mechanism both in higher plants (for a review see Ernst et al. 1992) and fungi (Ross 1993). Although the significance of the proposed mechanism is not clear in both fungi (Ross 1993) and higher plants (Ernst et al. 1992), a similar mechanism has been suggested to protect mycorrhizal roots from metal toxicity. It was suggested that sorption of metals to fungal tissues or intracellular uptake and detoxification in fungal vacuoles subsequently reduced metal uptake into the host plant (Brown and Wilkins 1985, Jones and Hutchinson 1986).

Although there is no doubt that metal sorption on fungal structures does occur, the extent to which it occurs and its significance to the mycorrhizal symbiosis are unclear. Most evidence for metal binding by fungal cell walls derives from biotechnological studies aiming at wastewater management. These investigations show that fungal cells possess a high metal adsorption capacity (Kapoor and Viraraghavan 1995). However, most studies have been conducted with *Aspergillus*, *Penicillium*, *Rhizopus* or other fungi, and not with

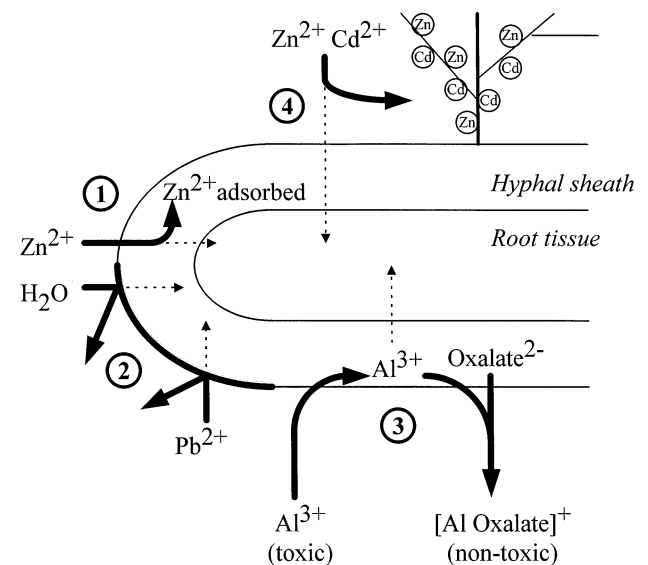


Fig. 1. Metal exclusion mechanisms possibly operating in ectomycorrhizas. (1) Sorption of metals in the hyphal sheath, (2) reduced apoplastic mobility as a result of hydrophobicity of the fungal sheath, (3) exudation of chelating substances, (4) sorption on the external mycelium.

higher fungi of the Basidiomycetes. This is important as cell wall composition and, thus potential binding sites vary with fungal taxonomic group (Wessels and Sietsma 1981, Gadd 1993). Data on metal sorption by ectomycorrhizal fungi are scarce. Marschner et al. (1998) measured Pb sorption on the mycelium of the ectomycorrhizal fungi *Paxillus involutus* and *Laccaria bicolor*. They found that Pb sorption after exposure to 48 μM Pb was as high as 0.2–1.3 mmol (g mycelial dry weight)⁻¹. As this amount was similar to the cation exchange capacity of these fungi, most of the Pb was probably bound to cation exchange sites, although dependent on the fungal species, some Pb may also be immobilised by precipitation (Marschner et al. 1998). In the study by Marschner et al. (1998), Pb sorption was measured under conditions promoting sorption with high concentrations of Pb and no other cations except protons present in significant concentrations in the loading solution. Under realistic conditions, cations compete for binding sites and, thus, the presence of other cations will substantially reduce Pb sorption (Marschner et al. 1998). This feature may strongly depend on the metal considered. Copper, for example, may show a much higher affinity to bind to fungal cell walls than Pb, although the relative affinity of different metals to bind to fungal cell walls depends on the chemical composition of the cell wall and, thus, the fungal species involved (Kapoor and Viraraghavan 1995). For mycorrhizal fungi, however, no detailed studies on sorption characteristics based on a range of metals are yet available.

In spite of the limited information on the sorption properties of mycorrhizal fungi, a comparison of metal concentrations in shoots of mycorrhizal and non-mycorrhizal plants has been taken as evidence for significant metal sorption on fungal tissues and restricted metal uptake into host plants. These studies, in many cases (but not all), have shown lower concentrations in shoots of mycorrhizal than in non-mycorrhizal plants. However, there are two major problems encountered with the use of shoot concentrations as a diagnostic indicator of root metal exposure.

(1) *Dilution*. Reduced shoot concentrations could be caused by dilution of the metal due to improved shoot growth. Recalculation of literature data provides evidence that in many cases, mycorrhizal fungi had little influence on the metal contents of shoots. In some cases, mycorrhizal fungi even increased the metal translocation to the shoots. Interestingly, investigations presenting clear evidence for amelioration of metal toxicity in terms of improved growth in many cases failed to show reduced metal translocation to the shoots (Table 1).

(2) *Altered transfer to the shoot*. Toxic metals may impair transpiration of plants by either interfering with stomatal regulation or reducing water uptake by the root system, or both (Barceló and Poschenrieder 1990). As at least part of the metals may be transported to the shoot via the transpiration stream, reduced transpiration may result in decreased translocation of metals to shoots. This creates a complicated situation for the interpretation of mycorrhizal effects on shoot contents, as transpiration-driven metal translocation may differ between non-mycorrhizal and mycorrhizal plants because of differences in metal intoxication. The situation becomes even more complex considering that translocation

to the shoot may also be affected by the hormonal status of the plant. Vodnik et al. (1999) showed that increased Pb tolerance of *Picea abies* seedlings induced by external supply of cytokinins to the roots was associated with decreased root but increased shoot contents of Pb. Although the mechanism(s) underlying phytohormone effects on metal distribution within the plant are not understood at present (Vodnik et al. 1999), the results clearly demonstrate that metal translocation to the shoot is not a simple, uncontrolled process in which a fixed proportion of metals taken up by the roots is delivered to the shoots. As mycorrhizal fungi alter the hormonal status of their hosts (Gogala 1991), it is likely that these changes in phytohormones may indirectly affect metal concentrations in the shoot in a presently unpredictable manner.

In summary, shoot concentrations and contents of Al or heavy metals are a poor measure of the degree of metal exposure of the root system, and are poorly related to amelioration of metal toxicity. Alternative approaches used to assess differences in metal uptake between mycorrhizal and non-mycorrhizal root systems include measurements of desorption kinetics of metals bound to the root system or of metal distribution using microanalytical techniques. In *Betula papyrifera* seedlings exposed to Ni, mycorrhizal colonisation by *Scleroderma flavidum* reduced the fraction of Ni exchangeable with Ca, indicating that *Scleroderma flavidum* did not increase metal sorption on the root system but in fact reduced it (Jones et al. 1988). In an experiment with Norway spruce seedlings loaded with Pb, similar biphasic desorption kinetics were found in both non-mycorrhizal and mycorrhizal roots (Jentschke et al. 1998), indicating little influence of mycorrhizas on apoplastic binding of metals. However, these methods cannot show exactly where the desorbed metals originated from. To assess the metal distribution in mycorrhizal root systems in a direct manner, microanalytical methods have been used by a number of investigators allowing differentiation between host plant and fungal metal uptake. In these studies, the preparation technique is critical in decreasing the redistribution of metals during tissue fixation, embedding and cutting of sections. In many published investigations (e.g. Turnau et al. 1993, 1996), however, conventional tissue fixation methods were used in which redistribution of ions is known to occur. For example, Strullu et al. (1982) showed that polyphosphates in fungal vacuoles were associated with Ca after conventional glutaraldehyde fixation, but, after cryofixation and freeze-substitution, P was found mainly associated with K and not Ca (Orlovich and Ashford 1993). Recently, Bücking and Heyser (1999) have confirmed these results. Although some intracellular uptake of Al into fungal cells of the fungal sheath (Jentschke et al. 1991b) and binding to polyphosphates in fungal vacuoles (Martin et al. 1994) may occur, the highest concentrations of Al were not found in fungal structures but in the root cortex when adequate techniques were used (Jentschke et al. 1991b). For Pb (Jentschke et al. 1991a) and Zn (Denny and Wilkins 1987b), similar results were found. These data suggest that adsorption of Al, Pb and Zn on fungal cell walls of the hyphal sheath or Hartig net is no stronger than that on host tissues. However, this may depend on the plant and fungal symbionts, the hydro-

Table 1. Shoot content of mycorrhizal plants exposed to Al or heavy metals, expressed as relative values. Total metal content in shoot compartments of non-mycorrhizal plants = 100%. *Metal concentration in soil ($\mu\text{mol kg}^{-1}$).

| Metal | Concentration in rooting medium (μM) | Fungus | Tree species | Metal content in shoot compartment | | Reference |
|---|---|---|--------------------------|------------------------------------|-------------------------------|--------------------------------|
| | | | | Part | (% of non-mycorrhizal plants) | |
| <i>Fungi showing amelioration of metal toxicity</i> | | | | | | |
| Al | 200 | <i>Pisolithus tinctorius</i> | <i>Pinus rigida</i> | Needles | 99 | Cumming and Weinstein (1990a) |
| | 460 | <i>Pisolithus tinctorius</i> | <i>Pinus strobus</i> | Needles | 148 | Schier and McQuattie (1995) |
| | 800 | <i>Paxillus involutus</i> | <i>Picea abies</i> | Needles | 89 | Hentschel et al. (1993) |
| Cd | 0.5 | <i>Paxillus involutus</i> | <i>Picea abies</i> | Needles | 154 | Jentschke et al. (1998) |
| | 34 | <i>Scleroderma flavidum</i> | <i>Betula papyrifera</i> | Stems | 80 | Jones and Hutchinson (1986) |
| Ni | 34 | <i>Laccaria proxima</i> | | Leaves | 125 | |
| | | <i>Lactarius hibbardae</i> | | Stems | 160 | |
| | | | | Leaves | 150 | |
| Cu | 32 | <i>Paxillus involutus</i> | <i>Pinus sylvestris</i> | Stems | 170 | |
| | | <i>Thelephora terrestris</i> | | Leaves | 140 | |
| | | | | Needles | 60 | VanTichelen et al. (1999) |
| No effect of mycorrhizal colonisation on metal sensitivity | 6000 | <i>Suillus bovinus</i> | <i>Pinus sylvestris</i> | Needles | 80 | |
| | | <i>Laccaria bicolor</i> | | Shoots | 100 | Göransson and Eldhuset (1991) |
| | | <i>Suillus luteus</i> | <i>Quercus rubra</i> | Needles | 96 | Jentschke et al. (1998) |
| Cd | 0.5 | | | Leaves | 40 | Dixon (1988) |
| | 178* | | | Stems | 160 | Jones and Hutchinson (1986) |
| Ni | 34 | <i>Lactarius rufus</i> | <i>Betula papyrifera</i> | Leaves | 190 | |
| | | | | Stems | 89 | Jones and Hutchinson (1986) |
| Cu | 32 | <i>Scleroderma flavidum</i> | <i>Betula papyrifera</i> | Stems | 185 | |
| | | <i>Laccaria proxima</i> | | Stems | 104 | |
| | | <i>Lactarius hibbardae</i> | | Stems | 85 | |
| Non-mycorrhizal and mycorrhizal seedlings not affected by metals supplied | 14 000 | <i>Thelephora terrestris</i> | <i>Pinus sylvestris</i> | Shoot | 122 | Colpaert and van Assche (1992) |
| | | <i>Laccaria laccata</i> | | Shoot | 89 | |
| | | <i>Scleroderma citrinum</i> | | Stems | 81 | |
| Zn | 3000 | <i>Paxillus involutus</i> | | Stems | 48 | |
| | | <i>Suillus bovinus</i> NP1 | | Stems | 79 | |
| | | <i>Suillus bovinus</i> P2 | | Stems | 40 | |
| | | not identified | <i>Pinus sylvestris</i> | Shoot | 100 | Bücking and Heyser (1994) |
| | | <i>Suillus bovinus</i> | | Shoot | 90 | |
| | | <i>Suillus bovinus</i> pretreated with Zn | | Shoot | 37 | |
| | | <i>Thelephora terrestris</i> | <i>Pinus sylvestris</i> | Needles | 35 | Colpaert and van Assche (1993) |
| Cd | 44.5 | <i>Laccaria laccata</i> | | Needles | 72 | |
| | | <i>Scleroderma citrinum</i> | | Needles | 42 | |
| | | <i>Paxillus involutus</i> | | Needles | 37 | |
| Pb | 5 | <i>Suillus bovinus</i> NP1 | | Stems | 30 | |
| | | <i>Suillus bovinus</i> P2 | | Stems | 27 | |
| | | <i>Suillus bovinus</i> P3 | | Stems | 26 | |
| 97* | 97* | <i>Paxillus involutus</i> | <i>Picea abies</i> | Stems | 16 | Marschner et al. (1996) |
| | | <i>Laccaria laccata</i> | | Leaves | 81 | |
| | | <i>Suillus luteus</i> | <i>Quercus rubra</i> | Leaves | 50 | Dixon (1988) |

phobic properties of their cell walls (see later), and/or on experimental conditions. Since the presence of mycorrhizas, in general, had little influence on the amount of metals bound to the cortex, the significance of metal sorption (Al, Zn, Pb) by hyphae of the fungal sheath or Hartig net for a reduction of uptake into host tissues is uncertain. The existing evidence, however, is based on the analysis of mature tissues in which it may be assumed that metals in the soil solution, in the cell wall solution, at the outer surface of the plasma membrane and both in the plant and fungal cell wall matrix are at equilibrium. Taking into account that metal uptake is a dynamic process, it is possible that sorption on fungal structures plays a role in reducing metal uptake when new tissue is produced during growth. Sorption could significantly reduce metal exposure at meristematic regions of the host plant, if the sorption capacity of the newly formed fungal tissue exceeds the influx of metals to the growing mycorrhizal tip. This has not been tested by model calculations of ion fluxes in the rhizosphere nor by experiments assessing metal distribution in apical regions of mycorrhizas. However, given the low elongation rate of mycorrhizal short roots, which may be more than 10-fold lower than that of non-mycorrhizal long roots, and given that the sorption capacity of the fungal tissues may not exceed that of host tissues, it is at present not clear whether this process significantly contributes to metal exclusion from apical zones of mycorrhizas.

Fungal hydrophobicity: restricted metal mobility in the fungal apoplast (exclusion mechanism 2)

Besides metal sorption to fungal cells, metal uptake into host tissues may be affected by the degree of hydrophilicity of the fungal apoplast. The fungal sheath formed by a hydrophobic fungus (Unestam 1991) could provide a barrier to apoplastic radial transport of water and ions. In fact, data by Ashford et al. (1988) and K. Turnau (unpublished, Table 2) support this hypothesis. The latter author recently investigated the metal distribution in mycorrhizal pine roots grown on industrial Zn wastes, using proton and electron microprobes of cryofixed and freeze-dried samples. She found that as compared to other mycorrhizas, mycorrhizas of *Rhizopogon roseolus* and *Suillus luteus* excluded Pb and

Table 2. Lead and zinc concentrations (mg g⁻¹ dry weight) in cortical cell walls or in the vascular system of pine mycorrhizas collected at a polluted site in Poland (K. Turnau, unpublished data).

| | Mycorrhiza | Cortical cell wall | Vascular system |
|----|----------------------------|--------------------|-----------------|
| Pb | <i>Rhizopogon roseolus</i> | 0.2 | 0 |
| | <i>Suillus luteus</i> | 0.4 | 0 |
| | <i>Hebeloma mesopheum</i> | 21 | 54 |
| | Ascomycete mycorrhiza | 28 | 142 |
| | | | |
| Zn | <i>Rhizopogon roseolus</i> | 0.4 | 1 |
| | <i>Suillus luteus</i> | 0.3 | 0 |
| | <i>Hebeloma mesopheum</i> | 8 | 42 |
| | Ascomycete mycorrhiza | 35 | 150 |
| | | | |

Zn from the root cortex and stelar tissues (Table 2). These data, for the first time, present evidence for a filtering effect of metals by certain mycorrhizal fungi. As both fungi effective in exclusion are hydrophobic, it is possible that restricted apoplastic water and ion mobility as a result of fungal hydrophobicity reduced metal transfer to root tissues. Yet, other mechanisms including adsorption on fungal tissues may also be involved. Interestingly, under laboratory conditions, the apoplast even of hydrophobic mycorrhizas was found to be permeable to ions such as La³⁺ (Behrmann and Heyser 1992) or Ca²⁺ and Mg²⁺ (A. Stettien, W. H. Schröder and A. J. Kuhn, unpublished results).

At present, it is not clear why results from field (Ashford et al. 1988, K. Turnau, unpublished) and laboratory grown mycorrhizas (Behrmann and Heyser 1992, Stettien et al., unpublished) are not consistent. Possible explanations include methodological problems as, for example, Ashford et al. (1988) used a dye as a tracer known to adsorb on chitin and cellulose (Behrmann and Heyser 1992). Also, rhizosphere effects may possibly have affected metal distribution in the mycorrhizas that Turnau collected from high-pH soil (7.4), as presumably metals had first to be mobilised in the rhizosphere before entering the mycorrhizas. Differences in the moisture regime between laboratory and field grown mycorrhizas (wet conditions in laboratory cultures vs. dry conditions in the field), which may have influenced the actual degree of water repellency by the hydrophobic fungi (Wessels 1997), may provide another likely explanation. Future work to unravel this problem should therefore include both laboratory and field material from a broad spectrum of fungal species and strains greatly differing in water repellency. The apoplastic ion mobility in these mycorrhizas should then be tested under both dry and wet conditions.

Chelation by organic acids and other substances released by mycorrhizal fungi (exclusion mechanism 3)

Increasing evidence suggests that exudation of organic acids (Jones 1998) plays a major role in Al tolerance of higher plants. In fungi, metal tolerance in some cases has been linked to extracellular chelation by organic compounds (Gadd 1993). Since mycorrhizal fungi exude a range of organic acids (e.g. Lapeyrie et al. 1987) or produce slime capable of binding metals, it is possible that organic compounds released by mycorrhizal fungi are responsible for the amelioration of metal toxicity in mycorrhizal plants. However, to date, investigations in this field are rare.

Ahonen-Jonnarh et al. (2000) recently showed that in contrast to non-mycorrhizal pine seedlings, seedlings colonised with *Suillus variegatus* or *Rhizopogon roseolus* responded to Al exposure with a strongly increased exudation of oxalic acid, which is an efficient Al chelator. Although these data do not prove that organic acids released by ectomycorrhizal fungi affect the metal sensitivity of the host plant, they highlight the potential role organic acids may have in the amelioration of metal toxicity by mycorrhizas.

Denny and Ridge (1995) found that excretion of fungal slime coincided with the tolerance of different strains of

Hymenoscyphus ericae, an ericaceous mycobiont, and with the degree of amelioration of Zn toxicity in *Calluna vulgaris* mycorrhizal with these strains. This correlation was apparent only for 'loosely adhering slime' (Denny and Ridge 1995) but not for slime strongly associated with fungal cell walls. Since the slime, which probably consisted of glycoproteins, strongly bound Zn from nutrient solutions in *in vitro* cultures of the fungi, the authors concluded that this slime may play a role in reducing the metal exposure to both the mycobiont and the host plant and, thus in increasing their metal tolerance. Although striking similarities exist between this process and the production of mucilage on non-mycorrhizal root tips, which is a mechanism to reduce metal exposure of the root tips by extracellular binding of metals (Marschner 1991), the data base is presently too small to draw general conclusions. More studies are needed to confirm that slime and mucilage production in mycorrhizal root systems contributes to increased metal resistance.

Metal sorption on the external mycelium (exclusion mechanism 4)

In recent years, much attention has been focussed on the extent to which the external mycelium reduces net metal exposure. The hypothesis is based on the observation that the efficiency of fungal strains to reduce Zn and Cd translocation to the shoot was correlated with the amount of external mycelium produced by these strains (Colpaert and van Assche 1992, 1993). In addition, Denny and Wilkins (1987b) and work from our group (Jentschke et al. 1991a) demonstrated that the external mycelium may bind more Zn or Pb than the hyphal sheath or Hartig net. However, direct evidence that the binding of metals to the mycelium sufficiently reduces metal uptake into the root tissues has not yet been presented. The significance of metal immobilisation on the mycorrhizal mycelium depends (1) on the binding capacity of the mycelium and (2) on the availability of metals in the rooting substrate. Only when (1) is of a similar order of magnitude or greater than (2), at least locally, can it be expected that metal binding by the mycelium has a significant effect on effective metal concentrations in the rhizosphere. The use of culture techniques with artificial substrates and a relatively low supply of heavy metals may be expected to facilitate the expression of this effect. Marschner et al. (1996), using sand culture with frequently added nutrient solution containing low concentrations of Pb (5 μM), found a time-dependent reduction of Pb uptake into cortical cell walls of Norway spruce seedlings mycorrhizal with *Paxillus involutus* but not with *Laccaria bicolor*. After long-term exposure to Pb, no reduction in Pb uptake was found (Marschner et al. 1996). Although, again, no direct evidence for an involvement of the mycorrhizal mycelium in the reduction of metal uptake was presented, the most likely explanation for the observed time course of Pb uptake is a saturation of hyphal binding sites for Pb with, over time, increasing metal supply.

As these studies have used artificial substrates, the relevance of mycorrhizal hyphal binding of metals under natural conditions is unclear. Based on heavy metal

concentrations found in ectomycorrhizas in a *Pinus sylvestris* stand in southern Norway and estimates on total fungal biomass by ergosterol in soil, Berthelsen et al. (1995) calculated the contribution of ectomycorrhizal fungal biomass to the total heavy metal content in the soil. These estimates suggested that Cu, which was present at a low concentration, was mainly located in fungal biomass, whereas for Cd and Zn, which were found in higher concentrations, metal contents in the fungal biomass contributed to 30–40% of the total metal content of the soil. Lead present in the fungal biomass was only 2% of total Pb in the soil. Ledin et al. (1999) have recently confirmed the role of soil microorganisms in metal sorption in soils. They found up to 38% of Cd and Zn accumulated in soil microorganisms. However, the contribution of ectomycorrhizal hyphae to the metal sorption capacity of the soil is unclear at present, as even fundamental data on the contribution of mycorrhizal hyphae to the total fungal biomass in organic layers of temperate and boreal forest soils are lacking. Whatever this contribution is, it is important to consider that many organic soil components have strong affinities to adsorb metals (Ledin et al. 1999), reducing the potential contribution of mycorrhizal hyphae. The possibly limited contribution of mycorrhizal hyphae to the total metal binding capacity of the soil may explain why a strain of *Paxillus involutus* tended to decrease Pb uptake into cortical cell walls in sand culture, but not when grown in natural forest humus (Jentschke et al. 1997). There is a need for more investigations critically testing the role of the external mycelium on metal sorption especially in soils.

Other possible mechanisms linked to the external mycelium

The form of N (NH_4^+ vs. NO_3^-) taken up by the roots has a marked influence on rhizosphere pH (Marschner et al. 1991). Strong acidification may occur during ammonium uptake both along roots (Marschner et al. 1991) or ectomycorrhizal hyphae (B. Brandes 1999. Thesis, Univ. of Göttingen, Germany). Results from experiments with compartment culture systems in which hyphae are isolated from roots (Brandes et al. 1998) suggest that a great portion of NH_4^+ uptake in mycorrhizal root systems may be due to hyphal uptake. Therefore, H^+ efflux from mycorrhizal roots linked to NH_4^+ uptake may be reduced and taken over by H^+ efflux from hyphae. In addition, H^+ efflux from mycorrhizal roots may possibly be reduced by changed patterns of cation/anion uptake (Bledsoe and Rygielwicz 1986). As acidification increases the desorption of heavy metals from soil surfaces (Ledin et al. 1999), it may be speculated that mycorrhizal roots, especially those with an intensive external mycelium, may be much less affected by this release of metals. As adsorption of metals may not only be significant in soils but also on artificial rooting substrates like perlite or sand, differences observed between mycorrhizal and non-mycorrhizal plants in metal sensitivity may, at least in part, be explained in terms of changed rhizosphere chemistry affecting metal desorption from substrate surfaces. However, since protons not only increase desorption from soil or other surfaces, but may also ameliorate metal toxicity by reducing binding of metals to uptake sites

(Kinraide et al. 1992), the situation is complex. Experimental data testing this mechanism are lacking so far.

The proliferation of mycorrhizal fungal hyphae into the surrounding soil strongly enhances nutrient acquisition (especially P and N) by the root system (Marschner and Dell 1994, Brandes et al. 1998), although this may depend on the physiological compatibility of the symbionts (B. Brandes 1999. Thesis, Univ. of Göttingen, Germany). Since the nutritional status of a plant may have a pronounced influence on the susceptibility to metal toxicity (Clegg and Gobran 1995), it is possible that the improved nutrition contributes to the higher metal tolerance of mycorrhizal plants. Again, similar to other proposed mechanisms, a dense external mycelium providing greater nutrient acquisition would confer a higher metal tolerance to the host plant. In support of this mechanism, Jentschke et al. (1999) recently found that from two fungi tested, only *Paxillus involutus* increased Cd tolerance of the host plant. This coincided with a superior P supply of these plants. However, again, there is a need to test this hypothesis on the basis of a much broader spectrum of mycobionts and hosts.

In addition to the mechanism outlined above, mycorrhizal fungi may help to maintain nutrient uptake (e.g. P) when metal ions (e.g. Al) reduce their availability by precipitation reactions. Cumming and Weinstein (1990c) demonstrated that in contrast to non-mycorrhizal *Pinus rigida* seedlings, seedlings mycorrhizal with *Pisolithus tinctorius* were able to use sparingly soluble $AlPO_4$ as a P source. This may account for the observed difference in Al response between mycorrhizal and non-mycorrhizal seedlings in the experiments carried out by Cumming and Weinstein (1990a,b) in which relatively high P concentrations in the nutrient solution may have led to $AlPO_4$ precipitation. However, interaction of Al with P nutrition may not always occur (Marschner 1991), and amelioration of Al toxicity in *Picea abies* seedlings by the mycorrhizal fungus *Paxillus involutus* did not involve an offset of Al-induced P limitation (Hentschel et al. 1993).

Hormonal status

Mycorrhizal fungi produce a range of phytohormones (Gogala 1991) or modify the phytohormone levels in their hosts indirectly, for example, by improved mineral nutrition. As phytohormones (especially cytokinins) may play a role in metal tolerance of vascular plants (Pan et al. 1989), the possibility exists that the alleviation of metal toxicity in mycorrhizal plants is (partly) mediated by changed phytohormone relations of the host plant. At present, this hypothesis is only supported by indirect evidence. For example, although Al reduced Mg concentrations in both non-mycorrhizal and mycorrhizal *Picea abies* seedlings to a similar level of deficiency, mycorrhizal colonisation delayed the onset of needle chlorosis by several weeks (Hentschel et al. 1993). A similar pattern was observed in response to Ni (Jones and Hutchinson 1988b) and Cd (Jentschke et al. 1999). Possibly, mycorrhizas increased Mg efficiency of the seedlings or delayed leaf senescence by enhanced cytokinin export from the root system. Measurements of phytohormone pools and fluxes in non-mycorrhizal and mycorrhizal

seedlings affected by metals may provide further insight into the interaction of mycorrhizas, phytohormones and (heavy) metals.

Conclusions and future research needs

Clear evidence shows that ectomycorrhizal fungi ameliorate metal toxicity in their hosts. However, the ameliorative capacity may depend on the fungal species or strain, the specific metal and its concentration, and speciation in the rhizosphere. Although there has been a large number of studies aimed at unravelling the mechanisms involved, little convincing evidence exists, which demonstrates how mycorrhizal fungi affect the metal sensitivity of their hosts. There is some recent evidence that certain ectomycorrhizal types may exclude metals from the host plant, although the mechanism responsible for this (exudation of organic acids, adsorption, intracellular sequestration or hydrophobic restriction of apoplastic mobility) is not clear at present. Binding to the external mycelium may be an important means by which metal exposure of the host plant is reduced, but direct evidence for the significance of this process, both in artificial substrates or in soils, is still lacking. Other possible mechanisms, including hormonal effects, have not been studied thoroughly in ectomycorrhizas. Metal chelation by organic substances released from mycorrhizal fungi has recently been demonstrated to be potentially important to ameliorate Al toxicity in ectomycorrhizal associations. In a number of cases, an increase in metal tolerance was associated with higher P levels in plants; thus improvement of the mineral nutritional status of the host plant alone may confer a higher resistance to metals.

In the past, most research has focused on metal immobilisation on or in fungal structures as a means of metal exclusion from the host plant. To complement this research, microanalytical studies using appropriate techniques should measure short-term uptake and exchange kinetics of metal ions within the apoplast of mycorrhizas in comparison to non-mycorrhizal roots with special regards to meristematic regions. Although intracellular uptake across the plasma membrane is difficult to estimate, secondary ion mass spectrometry (SIMS) has the potential to do so (Lazof et al. 1996). This could show whether, or at what structural level, mycorrhizas may exclude metals from uptake. Further research should then try to unravel the reasons for metal exclusion (e.g. exudation of organic acids, adsorption on fungal tissues, hydrophobicity of fungal cell surfaces). To critically test the role of adsorption, it would be helpful to develop dynamic models simulating metal fluxes in the rhizosphere and apoplast of mycorrhizas. This could show whether the sorption capacity of newly formed fungal tissues exceeds the influx of metals to the growing mycorrhizal tip. To evaluate the role of the external mycelium in immobilisation of metals, experiments should be set up which quantify metal fluxes in the growth units and estimate the absolute amount of metals immobilised on fungal structures in comparison to the amount of metals taken up by the plants. These experiments should also include the use of soil as the rooting substrate.

The role of hormones could be studied using mycorrhizal mutants or genetically modified strains showing different levels of phytohormone production.

Up to now, almost all published studies are based on long-term experiments (weeks to months). Given the large spectrum of possible mechanisms, short-term experiments (hours to days) could help to identify primary targets of mycorrhizal benefits in metal tolerance (e.g. short vs. long root growth). The advantage of short- over long-term experiments is that they are not confounded by effects on mineral nutrition of the plants, thus allowing separation of direct effects on root growth from indirect effects via altered mineral nutrition.

As a final step, there is a need not only to determine the function of potential mechanisms under laboratory conditions using few model organisms, but also to ascertain the ecological significance of these mechanisms working in a real soil environment and considering the broad range of mycorrhizal fungi present in forest ecosystems.

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