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Effects of mycorrhizal colonisation on *Thymus polytrichus* from heavy-metal-contaminated sites in northern England

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Abstract A study was performed to establish whether colonisation with arbuscular mycorrhizal (AM) fungi is beneficial to wild thyme [*Thymus polytrichus* A. Kerner ex Borbás ssp. *britannicus* (Ronn.) Kerguelen (Lamiaceae)] growing in the heavy-metal-contaminated soils along the River South Tyne, United Kingdom. *T. polytrichus* plants of the same genotype were grown under controlled conditions with and without Zn contamination, and differences between AM-colonised and -uncolonised plants in mean shoot and root growth (dry weight) and Zn concentration were assessed. When grown in the heavy-metal-contaminated, low-P soil from one of the South Tyne sites, AM-colonised plants grew significantly larger than uncolonised plants; however, there was no significant difference in growth between AM and non-AM plants grown in an artificial substrate with a larger available P concentration, with or without Zn contamination. Mycorrhizal colonisation increased tissue Zn concentrations during the experiments. It is concluded that AM fungi are beneficial, if not essential, to *T. polytrichus* growing in the low-nutrient soils along the River South Tyne, because of their role in enhancing plant uptake of P (and possibly other nutrients). There was no evidence from this study that the fungi reduce plant uptake of heavy metals at these sites, but rather increase Zn uptake. However, the resulting tissue metal concentrations do not appear to be large enough to be detrimental to plant growth.

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Introduction

An earlier study (Whitfield et al. 2003) showed that the roots of wild thyme [*Thymus polytrichus* A. Kerner ex Borbás ssp. *britannicus* (Ronn.) Kerguelen (Lamiaceae)] growing in the heavy-metal-contaminated soils along the River South Tyne in northern England are generally well colonised by arbuscular mycorrhizal (AM) fungi. This could imply that the plants derive some benefit from the mycorrhizal symbiosis (Sanders and Fitter 1992), raising the possibility that AM colonisation contributes to host metal tolerance at these sites (see Whitfield et al. 2003).

A number of studies have demonstrated beneficial effects of AM colonisation on plants growing in metal-contaminated substrates, but these effects were not consistent between studies. In some experiments colonisation both reduced host tissue metal concentration and increased biomass production (El-Kherbawy et al. 1989; Heggo et al. 1990; Weissenhorn et al. 1995; Chen et al. 2003), but in others host growth was enhanced without any apparent reduction in metal uptake (Dueck et al. 1986; Heggo et al. 1990; Davies et al. 2001), or host metal concentrations were reduced without any detectable growth benefits (Gildon and Tinker 1983; Li and Christie 2000). In some experiments AM colonisation in fact appeared to increase metal toxicity to the host (Killham and Firestone 1983; Galli et al. 1995; Weissenhorn et al. 1995).

The objectives of the present study were to confirm, using controlled experiments, whether AM colonisation enhances the growth of *T. polytrichus* in the heavy-metal-contaminated soils along the River South Tyne, and to assess whether such effects are mediated simply by enhanced nutrient uptake, or by reduced uptake of toxic metals.

Materials and methods

Experiment 1

Sections from two *T. polytrichus* clones were collected from two different heavy-metal-contaminated sites along the River South Tyne (see Whitfield et al. 2003). After removing most of the soil from the roots, the plants were grown in 15 cm pots of uncontaminated peat-based compost in an unheated greenhouse for several months, with supplementary lighting using 400 W mercury vapour lamps to maintain a daylength of 16 h. Subsequent testing of the relative rate of root growth of cuttings from the two clones in 0.5 g l^{-1} $Ca(NO_3)_2$ before and after addition of Zn (Wilkins 1957) showed that one clone (HT) had a relatively high and one (LT) a relatively low Zn tolerance index (TI: 42% and 2.5% respectively).

Similar-sized cuttings were taken from the two clones and allowed to root in 0.5 g l^{-1} $Ca(NO_3)_2$ solution for 12 days before being blotted and weighed; the length of the longest root was then measured (mean±SE length of longest root: HT 18.1±1.16 mm; LT 33.0±1.45 mm). The fresh weights and root lengths were used to estimate the initial dry weight of each shoot, based on measurements made on ≥20 rooted cuttings from the same plants weighed before and after oven-drying (calculated mean±SE shoot dry weights: HT 5.26±0.07 mg; LT 6.72±0.15 mg).

Dried, sieved (<2 mm) soil samples from heavy-metal-contaminated site M3 collected and analysed earlier (see Whitfield et al. 2003) were pooled and mixed thoroughly. The soil was then sterilised by heating to 100°C for 2 h on three consecutive days to kill any viable mycorrhizal spores and hyphae, as well as other potential sources of plant infection (pathogenic fungi, bacteria, etc.). The mycorrhizal inoculum used for the experiment consisted of a carrier material (a mixture of expanded clay granules containing zeolite, argillite and attapulgite) containing spores and root fragments from monocultures of *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, *G. caledonium* (Nicol. & Gerd.) Trappe & Gerd., *G. claroideum* Schenck & Smith, and *G. intraradices* Schenck & Smith, all originating from heavy-metal-contaminated mine spoils (first three: La Banque Européenne de Glomales (BEG) isolates 132, 133 and 134 respectively; BEG number not yet allocated to the *G. intraradices* isolate). (Inoculum and carrier material supplied by PlantWorks UK, Sittingbourne, Kent, UK.) The inoculum was added (1:10 v/v) to half of the soil, and the carrier material alone added to the other half (control treatment), and mixed thoroughly. Two subsamples of the soil/carrier mix were used to measure the EDTA-extractable Pb and Zn [Ministry of Agriculture, Fisheries and Food (MAFF) 1986a], and three subsamples to measure the $NaHCO_3$-extractable P concentration (MAFF 1986b). The results and a summary of the soil characteristics for site M3 are given in Table 1.

Twenty-four 6 cm plastic plant pots were filled with the inoculated soil and 24 with the control soil, and the soils soaked thoroughly with tap water. One cutting was planted in each pot (12 HT and 12 LT per soil treatment), and a thin layer of coarse grit spread over the soil surface to prevent contamination of control pots with inoculum. The pots were laid out randomly in an unheated greenhouse (mean minimum/maximum daily temperatures: 16/26°C), with supplementary lighting as described above, and watered daily with tap water. As the plants in the AM treatment appeared healthy with no signs of nutrient deficiency throughout this period, no supplementary feeding was given. After 66 days the whole plants were removed carefully from the pots, washed with tap water followed by distilled water, and the roots and shoots separated. The roots were blotted on absorbent paper and weighed, then the distal part of the root system (approximately 30–40% of the whole root) was removed and stored in 50% ethanol at 5°C for mycorrhizal analysis. The remaining roots were re-weighed, and the partial root and whole shoot samples dried overnight in paper bags at 70°C, then stored in a desiccator. The dried tissues were weighed, and the increase in shoot dry weight calculated for each plant. The root sample dry and fresh weights were used to calculate the approximate dry weight of the whole root: this was considered a good approximation of root dry weight increase, as the dry weight of the roots at the start of the experiment was negligible.

Shoot and root tissues were digested using the following method, adapted from that of Andrews et al. (1989), and analysed for total P and Zn concentrations. (Preliminary analyses had shown that shoot concentrations of the other contaminating metals, Cd and Pb, were too low to be measured accurately in samples of this size: typical Cd and Pb concentrations in shoots from site M3 were <1.2 and <30 mg kg^{-1} respectively.) The tissues were cut into 1–2 mm fragments, and larger samples (>120 mg) divided into two equal-sized portions. Each sample was loaded into a 10 ml digestion tube, weighed and 2 ml concentrated nitric acid was added. The tubes were fitted with condensers, and the samples refluxed in a thermostatically controlled heating block at 140°C for 3 h.

The cooled digests were decanted into volumetric flasks, and deionised water was used to wash out the digestion tubes and to make up the volume of each sample to exactly 25 ml. The samples were filtered through Whatman No. 40 ashless filter papers, and the concentration of Zn in the solutions measured using a Unicam 929 atomic absorption spectrometer (Unicam Analytical Instruments, Madison, Wis.); these measurements and the tissue weights were used to calculate tissue Zn concentrations. Tissue P concentrations were measured by reaction of a known volume of the digest with acid ammonium molybdate (MAFF 1986b), and measurement of the intensity of the resulting blue colour at 880 nm using a Unicam 8625 UV/VIS spectrometer. Results from duplicate samples were averaged.

The distal root samples were cleared and stained with acid fuchsin as described earlier (Whitfield et al. 2003), and small subsamples of each were examined under ×200 magnification for presence or absence of AM colonisation, including arbuscules. Percentage root length of the AM-inoculated plants colonised by AM fungi was assessed by a gridline intersect method (Giovannetti and Mosse 1980): the entire root sample was cut into 1–2 cm sections and distributed evenly across a Petri dish marked with a 1 cm grid, and a dissecting microscope used to assess percentage AM colonisation, based on the number of root/gridline intersections (out of 100) containing AM hyphae.

To control for any effects of root fragments in the AM inoculum on soil properties and hence plant growth, the non-mycorrhizal treatments should ideally have contained sterilised inoculum (George 2000). As there was insufficient inoculum for this purpose, at the end of the experiment the AM-inoculated soil was remixed, half of the soil sterilised (as above), and the experiment repeated

Table 1 Soil characteristics, experiment 1 (concentrations expressed as proportion of oven-dried weight)

	Site M3 (mean)[a]	Soil/carrier mix (mean±SD)
Extractable Cd (mg kg^{-1})	2.50	nd[b]
Extractable Pb (mg kg^{-1})	525.4	531.0±18.63
Extractable Zn (mg kg^{-1})	616.5	496.1±13.43
Solution Zn (mg l^{-1})	0.26	nd
Extractable P (mg kg^{-1})	2.86	4.61±0.20
Total C (g kg^{-1})	22.66	nd
pH	6.9	nd

[a] See Table 1 in Whitfield et al. (2003) for full analysis
[b] Not determined

using 12 cuttings only, from the same high-tolerance clone as above, half planted in the unsterilised and half in the sterilised soil (calculated mean±SE shoot dry weight: 3.89±0.14 mg). Growth conditions were as above, and the plants were harvested after 93 days.

Experiment 2

Because the soil used in experiment 1 had a very low P content, it was possible that any improvement in growth resulting from AM inoculation could be caused simply by enhanced nutrient uptake, rather than direct effects on metal toxicity. In addition, the lack of a control soil treatment (without heavy metals) meant that it was not possible to show whether the concentrations of metals present caused a reduction in growth of non-mycorrhizal plants, i.e. demonstrable toxicity. The experiment was therefore repeated using an artificial growth medium with a slightly higher available P content than the substrate used in experiment 1, and with four treatments: a mycorrhizal and a non-mycorrhizal treatment in uncontaminated (control) substrate, and the two treatments in the same substrate spiked with $ZnSO_4$ (see below).

The growth medium used was a mixture of equal volumes of sterile silver sand (Silvaperl, Sinclair, Gainsborough, Lincs, UK) and an attapulgite clay soil conditioner (Agsorb 8/16, Oil-Dri, Wisbech, Cambs, UK). This mixture has been demonstrated to promote AM colonisation in trap cultures with various hosts, and in preliminary growth trials both mycorrhizal and non-mycorrhizal *T. polytrichus* cuttings grew equally vigorously for several weeks, indicating an adequate supply of nutrients. The dry components were mixed, and half of the mixture used to fill 22 lined 6 cm plant pots, and spiked with 1,000 mg l^{-1} $ZnSO_4$ by standing the pots in the solution for a few seconds until soaked through (resulting substrate Zn concentration approximately 500 mg kg^{-1} dry weight, calculated from mean volume of solution taken up by a known weight of substrate).

Previous trials had shown that soil solution Zn concentrations immediately after soaking this mixture in $ZnSO_4$ were high (>20 mg l^{-1}), and declined over several weeks to concentrations similar to those found in soil from field site M3. Thereafter, the concentration tended to fluctuate, but remained below 1 mg l^{-1}, with an overall decline to 0.1–0.2 mg l^{-1} over 8–10 weeks. The substrate was therefore kept moistened with distilled water for 4–6 weeks to allow the Zn concentration in the soil solution to equilibrate. Porous soil solution samplers (Menzies and Guppy 1999) in two of the spiked pots were used to monitor soil solution Zn concentrations, and cuttings planted when concentrations fell below 1.0 mg l^{-1}.

The Zn-contaminated mixtures were pooled and remixed just before planting, and mycorrhizal inoculum added to half of the uncontaminated and half of the contaminated substrate. The inoculum used in this experiment consisted of washed, finely chopped fresh roots of *T. polytrichus* plants grown from pre-rooted cuttings in a sterile sand/Agsorb mixture (as above, with a small amount of bone meal to provide slow release of P), into which had been mixed roots and soil from one of the AM-colonised *T. polytrichus* clones transplanted earlier from site M3. (This technique can be problematic in that plants inoculated in this way can become infected with pathogenic fungi; however, in the experiment reported here the inoculated roots were later found to be well colonised with AM fungi, and appeared healthy and free of obvious pathogens.)

Ten 6 cm pots were filled with AM-inoculated substrate and 10 with uninoculated unspiked substrate, and 11 pots with each of the Zn-spiked mixtures: one of the pots from each of the Zn treatments was used for soil solution sampling (as above) and the plants in those pots were not included in the results. Similar-sized *T. polytrichus* cuttings (mean±SE shoot dry weight 7.45±0.17 mg, maximum root length 21.7±1.40 mm) from the same LT clone used in experiment 1 were planted in the pots as described above. The pots were laid out randomly on individual plastic trays in a growth room, and maintained on a constant temperature/light cycle (20±2°C day, 15±2°C night; 16 h daylength; mean irradiance at plant level 2.75 W m^{-2}), and bottom-watered with distilled water every 3 days. After 4 weeks the shoots in all treatments were beginning to look slightly chlorotic, so a weak complete-nutrient solution (0.2 g l^{-1} Chempak Formula 3; Chempak Products, Hoddesdon, Herts, UK) was supplied every 6 days from day 30 until the end of the experiment.

Soil solution samples were collected from the two Zn-contaminated pots approximately 20 min after each watering, and the Zn concentration in the samples measured by atomic absorption spectrometry. The plants were harvested after 68 days and analysed as above. Analysis of three subsamples of the uninoculated Zn-treated substrate at the end of the experiment showed an extractable P concentration (mean±SD) of 10.0±0.65 mg kg^{-1}.

Stained root samples from all plants were again examined under ×200 magnification to confirm presence or absence of AM fungi, but detailed counts were performed only on the roots from the Zn-treated inoculated plants.

Experiment 3

Experiment 2 was repeated using a larger Zn concentration (pots soaked in 1,500 mg l^{-1} $ZnSO_4$ solution; calculated substrate Zn concentration ca. 750 mg kg^{-1} dry weight). The mycorrhizal inoculum used was the same as that in experiment 1, with carrier material added to the control pots. The experiment was replicated using both of the *T. polytrichus* clones used in experiment 1, and the plants harvested and analysed after 52 days. As the plants appeared to be growing slowly (presumably because of Zn toxicity), plants were watered with 0.4 g l^{-1} Chempak (as above) every 3 days from day 36. The extractable P concentration (mean±SD, three subsamples) in the uninoculated Zn-treated substrate at the end of the experiment was 18.0±1.93 mg kg^{-1}.

Statistics

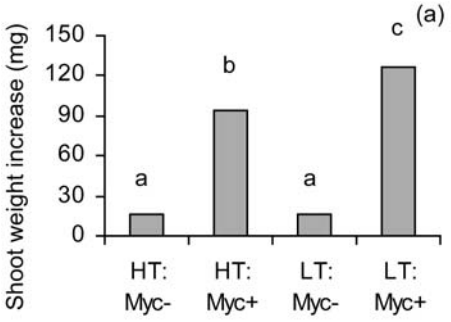
Root and shoot dry weights and tissue P and Zn concentrations were log-transformed, and shoot:root Zn and P ratios arcsine-transformed, where necessary to fit the data to a normal distribution (assessed by the Kolmogorov-Smirnoff test). Significance of differences between treatments was assessed using one-way analysis of variance (ANOVA) with least significant difference (LSD) tests, or pairwise by Student's *t*-test if variances could not be homogenised. All analyses were performed using the SPSS statistical package, release 10 (SPSS, StatSoft, Cary, N.C.).

Results

Experiment 1

The AM-inoculated roots were very well colonised, with 87–94% (mean 90%) of the root length colonised by hyphae, and arbuscules and vesicles present in abundance. Uninoculated roots were not colonised by AM fungi.

Shoot and root growth were very significantly greater in the AM plants in experiment 1 ($P<0.0001$; Fig. 1), the uninoculated plants being stunted and discoloured, with a tendency to shed the lower leaves. There was no significant difference in mean root weight between the HT and LT clone with either treatment, or in shoot weight increase in the non-AM plants, but shoot weight increased significantly more in the LT than in the HT clone when colonised by AM fungi ($P<0.05$). The follow-up experiment using sterilised AM-inoculated soil as the control



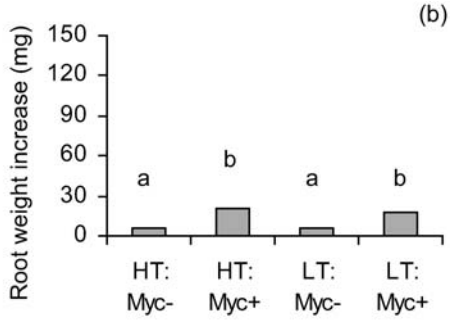
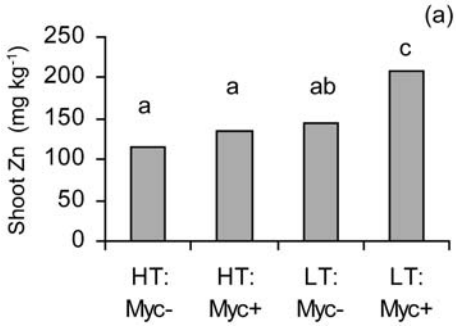


Fig. 1a, b Mean tissue dry weight increases, experiment 1: values labelled with the same letter are not significantly different ($P<0.05$, ANOVA). **a** Shoot weight increase: $F_{3,42}=92.3$, $P<0.0001$; *a* vs *b*/*c*, $P<0.0001$; *b* vs *c*, $P<0.05$. **b** Root weight increase: $F_{3,42}=40.9$, $P<0.0001$; *a* vs *b*, $P<0.0001$. In all figures, HT, LT indicate high- and low-tolerance clones; Zn+, Zn−, Myc+, Myc− indicate treatments with or without Zn/mycorrhizal inoculum



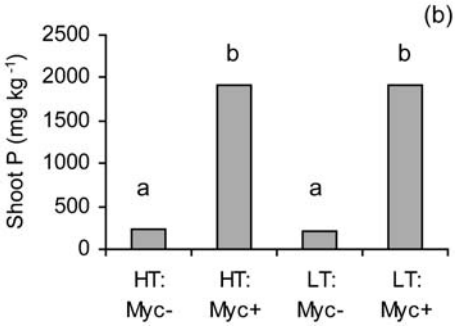


Fig. 2a, b Mean shoot Zn and P concentrations, experiment 1: values labelled with the same letter are not significantly different ($P<0.05$). **a** Shoot Zn: $F_{3,42}=11.33$, $P<0.0001$, ANOVA; *a* vs *c*, $P<0.0001$; *ab* vs *c*, $P<0.001$. **b** Shoot P: *a* vs *b*, $P<0.0001$, *t*-test; *n* for the four treatment groups, left–right: 6, 12, 6, 11

treatment produced similar results (mean shoot weight increases, AM and non-AM plants: 210.6 and 48.27 mg, $P<0.005$), indicating that growth was not increased simply by the root tissue in the inoculum.

The roots of the non-mycorrhizal plants in experiment 1 were too small to provide enough tissue for chemical analysis, therefore only shoot Zn and P concentrations are reported here. Mean Zn concentrations were larger in the mycorrhizal than in the non-mycorrhizal shoots, significantly so for the LT clone ($P<0.001$, Fig. 2a). Mycorrhizal colonisation very significantly increased P uptake into both clones ($P<0.0001$, Fig. 2b).

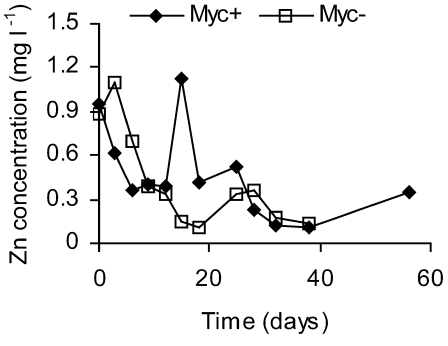


Fig. 3 Solution Zn concentrations in the growth substrate during experiment 2

Experiment 2

The mean solution Zn concentrations in the Zn-spiked substrate recorded during experiment 2 are shown in Fig. 3. As expected, the Zn concentrations fluctuated slightly over time but were always similar to the concentration found in metal-contaminated soil from site M3 (see Table 1). Concentrations in the AM-inoculated and uninoculated pots were very similar for most of the experiment. The AM-inoculated roots were again very well colonised, with 71–83% (mean 76%) of the root length of inoculated Zn-treated plants colonised by hyphae, and arbuscules and vesicles present in abundance. Uninoculated roots were not colonised.

In contrast to experiment 1, there was no significant difference in shoot or root weight increase between the AM and non-AM treatments, with or without Zn (Fig. 4). Both shoot and root concentrations of Zn were significantly larger in the mycorrhizal than in the non-mycorrhizal plants ($P<0.0001$; Fig. 5a). Root Zn concentrations were much larger than shoot concentrations, however,

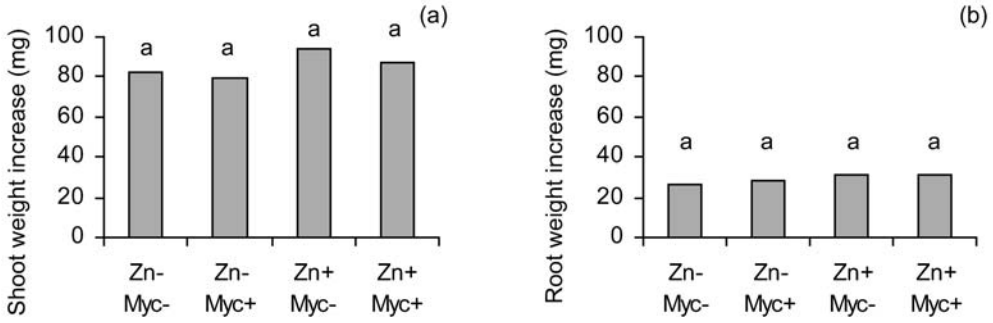


Fig. 4a, b Mean tissue dry weight increases, experiment 2: values labelled with the same letter are not significantly different ($P<0.05$, ANOVA). **a** Shoot weight increase: $F_{3,36}=2.16$, not significant (NS). **b** Root weight increase: $F_{3,36}=2.06$, NS

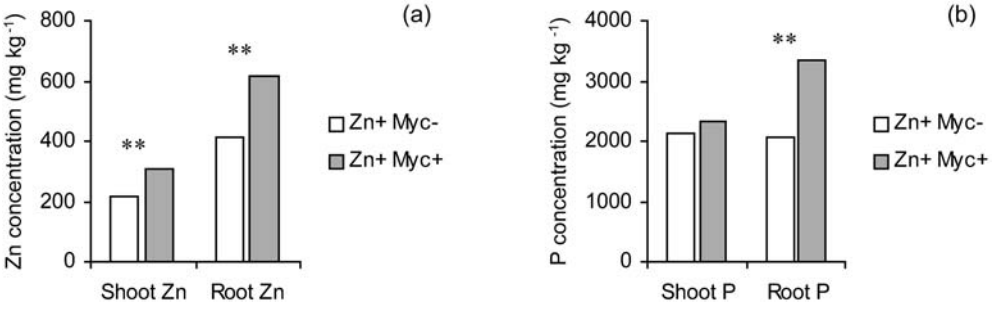


Fig. 5 **a** Mean tissue Zn and **b** mean tissue P concentrations, experiment 2 ($n=9$); $^{**}P<0.0001$, *t*-test

with mean root:shoot Zn ratios in the mycorrhizal and non-mycorrhizal plants of 2.00 and 1.94 ($P=0.68$, *t*-test).

Net P uptake into the root was greater in AM-colonised than in the uncolonised plants (mean concentration: 3,350 vs 2,076 mg kg^{-1}; $P<0.0001$, Fig. 5b), but shoot concentrations in the two treatments were very similar (2,338 vs 2,140 mg kg^{-1}; not significant).

Experiment 3

Solution Zn concentrations in the AM-inoculated and uninoculated pots were very similar for most of the experiment (Fig. 6). Only four measurements were made during this experiment, but solution Zn concentrations were clearly much higher than those in experiment 2 during the earlier part of the experiment. Many of the LT plants in experiment 3 died and this group was therefore not analysed further. The AM-inoculated Zn-treated HT plants were less well colonised than plants in the first two experiments (26–52%, mean 36%). However, plants grown without Zn in experiment 3 appeared only slightly better colonised, although percentage root length colonised was not measured.

As in experiment 2, there was no significant difference in mean biomass increase between the Zn-treated AM and non-AM plants, or in root weight between the two control (no Zn) treatments, while the AM plants in the Zn-free substrate had a slightly, but just significantly ($P<0.05$), smaller shoot weight increase than the control plants (Fig. 7). The Zn-treated plants, however, showed significantly less shoot and root growth than the controls.

Tissue Zn concentrations in this experiment were much larger than in experiments 1 and 2 (mean shoot Zn

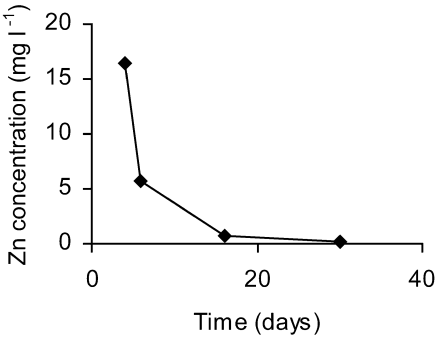


Fig. 6 Solution Zn concentrations in the growth substrate during experiment 3

in non-mycorrhizal plants: 1,099 mg kg^{-1}; Fig. 8a), but Zn concentrations in the root and shoot were very similar, with no significant difference in mean root:shoot Zn ratio between the mycorrhizal and non-mycorrhizal treatments (0.97 vs 0.91; $P=0.62$, *t*-test). The mycorrhizal plants again had higher mean tissue Zn concentrations than the non-mycorrhizal ones, significantly so for the roots ($P<0.05$).

The mycorrhizal plants had a greater mean root P concentration than the uninoculated plants (2,137 vs 1,733 mg kg^{-1}; $P=0.047$), with similar mean shoot concentrations (3,206 vs 3,249 mg kg^{-1}; not significant) (Fig. 8b). However, shoot P concentrations were much greater than root concentrations in this experiment (mean root:shoot P ratios 0.67 and 0.53 for the mycorrhizal and non-mycorrhizal treatments respectively), with far larger shoot and smaller root P concentrations than those in experiment 2.

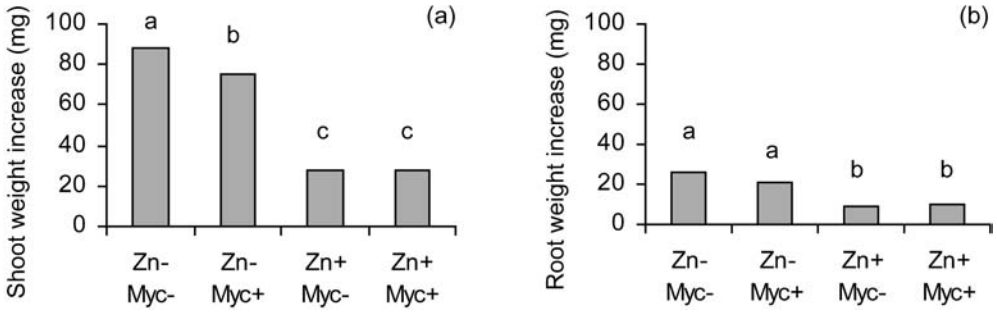


Fig. 7a, b Mean tissue dry weight increases, experiment 3: values labelled with the same letter are not significantly different (P<0.05). **a** Shoot weight increase: $F_{3,36}$=165.2, P<0.0001, ANOVA; *a*/*b* vs *c*, P<0.0001; *a* vs *b*, P=0.046. **b** Root weight increase (n=10): *a* vs *b*, P<0.0001, *t*-test

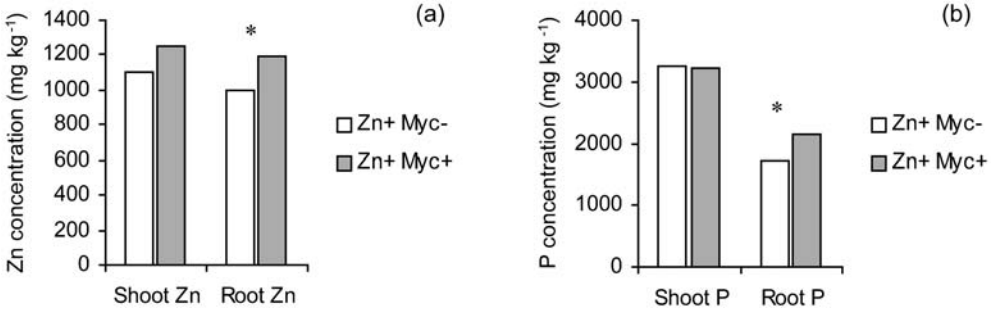


Fig. 8 **a** Mean tissue Zn and **b** mean tissue P concentrations, experiment 3 (n=8); *P<0.05, *t*-test

Discussion

Experiment 1 in this study demonstrates that *T. polytrichus* plants growing in the metal-contaminated alluvial sediments along the River South Tyne clearly benefit from AM colonisation. This effect appears to be mediated principally by improved nutrient uptake, demonstrated by the fact that the non-mycorrhizal plants in experiment 2, grown with adequate available nutrients, grew at least as well as mycorrhizal plants, in spite of having larger tissue Zn concentrations than those in the first experiment.

In fact, the mean root and shoot biomass increases in AM-inoculated plants were in some cases slightly reduced compared with the controls. However, this may simply represent a temporary "establishment cost" of AM colonisation: preliminary trials demonstrated that root colonisation under these conditions only begins 7–14 days after planting, and there is thought to be an establishment phase before the mycorrhizal symbiosis becomes functional, during which the fungi absorb photosynthates without supplying nutrients to the host in return (Kaldorf et al. 1999). This is supported by the finding that the smaller shoot growth in AM than in non-AM plants was only significant in experiment 3, in which the growth period was only 52 days; the AM plants in experiment 2, grown for 68 days, did not show this reduction. The shorter growth period may also account for the lower level of AM colonisation in experiment 3, although the increased P supply in that experiment may also have suppressed AM colonisation.

The effects of AM colonisation on host tissue concentrations of toxic metals have been shown to vary with the host (Díaz et al. 1996; Hildebrandt et al. 1999) and fungal species (Gildon and Tinker 1983; Shetty et al. 1995; Kaldorf et al. 1999), and with phytoavailable metal concentration. Dehn and Schüepp (1989), for example, found that AM colonisation of *Lactuca sativa* increased shoot Zn concentrations when substrate metal concentrations were small, but decreased shoot Zn when large concentrations were supplied (EDTA-extractable concentrations: 8.7 and 309.7 mg kg^{-1} respectively).

In some cases (e.g. Hildebrandt et al. 1999) AM colonisation has been shown to reduce both root and shoot metal concentrations, indicating reduced net uptake into the plant. The mechanism of this effect is unknown, but is thought (e.g. Dueck et al. 1986; Joner et al. 2000) to involve binding of the metals by the extraradical hyphae. Other studies have demonstrated increased root concentrations of metals in AM-colonised plants, but reduced translocation to the shoot, resulting in smaller shoot concentrations (Dehn and Schüepp 1989; Ricken and Höfner 1996; Chen et al. 2003) or higher root:shoot ratios (Joner and Leyval 1997). These results would suggest that while the mycorrhizal fungi increased metal uptake, they also caused sequestration of the metal in the root. Little is known about the mechanisms involved, but it has been proposed (e.g. Bradley et al. 1982) that the intraradical hyphae may provide binding sites for excess metals.

In the present study AM colonisation appeared to increase both root and shoot concentrations of Zn in *T. polytrichus* at all substrate concentrations tested. The

available Zn concentration in experiment 2 was not large enough to be toxic to the host; in experiment 3, however, the Zn supplied clearly was toxic, reducing plant growth significantly and killing many of the LT plants, but AM colonisation did not restore host growth. The root:shoot Zn ratio in the mycorrhizal plants was slightly higher than that of the controls, possibly indicating increased sequestration of metal, but overall there was little evidence of any direct reduction of metal toxicity from these experiments.

Given the differential effects on host growth and metal concentrations of different mycorrhizal species and strains indicated by previous studies, it is possible that other AM fungi isolated from *T. polytrichus* growing on the highly contaminated South Tyne shingles would be capable of reducing host tissue metal concentrations. Measurement of Zn concentrations in mycorrhizal *T. polytrichus* plants (mean colonisation rate 60–65%) growing at these sites showed that plants from site M3 had a mean shoot Zn concentration of 366 mg kg^{-1} (Whitfield 2002): this is even higher than the shoot concentrations found in experiments 1 and 2, suggesting that none of the other native AM fungi at this site reduce shoot metal concentrations. However, plants from a much more contaminated South Tyne site (site M4; mean soil extractable Zn concentration 937 mg kg^{-1}) had a lower mean shoot:soil Zn ratio (0.56 vs 0.69; Whitfield 2002); although other soil or host properties may have affected Zn uptake, this reduction could have resulted from sequestration by AM fungi. This hypothesis is supported by the study reported by Whitfield et al. (2003), in which some AM strains found in *T. polytrichus* roots from a heavily Zn-contaminated site were not found at the less contaminated sites studied.

Mycorrhizal colonisation is clearly important to *T. polytrichus* in terms of P uptake, experiments 1 and 2 demonstrating that the available P concentrations in the contaminated South Tyne soils are growth-limiting in plants of this species without mycorrhizas. An interesting finding from this study was the dramatic change in root:shoot P ratio with substrate Zn concentration. In experiment 2, in which non-toxic amounts of Zn were supplied, root P was similar to shoot P concentration in the non-mycorrhizal plants and greater than shoot P in the AM-colonised plants. In the Zn-treated plants in experiment 3, however, shoot P was much greater than root P concentration, and some 1.5 times greater than shoot P in the other two experiments. One possible explanation for these findings is that P is directly involved in Zn detoxification by the host (Van Steveninck et al. 1987, 1992; Davies et al. 1991), leading to an increased P requirement in the presence of large shoot metal concentrations. If this is the case, AM colonisation makes an important contribution to metal detoxification by increasing P uptake in this species.

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