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Uptake of Zn by arbuscular mycorrhizal white clover from Zn-contaminated soil

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Abstract

A randomised block glasshouse pot experiment compared the growth and Zn uptake of mycorrhizal and non-mycorrhizal white clover plants grown in a sterile soil/sand mixture containing 25 mg Zn kg⁻¹ to which five application rates of Zn (as ZnSO₄) from 0 to 400 mg kg⁻¹ were made. Two mycorrhizal inocula infected roots from the field and from clover trap cultures, were compared. Mycorrhizal infection (ranging from 33% to 46% of total root length) and Zn application had little effect on plant growth. Increasing Zn application rate led to increased uptake of Zn in roots and shoots (especially roots), but the increases were significantly greater in non-mycorrhizal controls than in mycorrhizal treatments. In contrast, P uptake was higher in mycorrhizal than in non-mycorrhizal plants. Plants that received trap culture inoculum had significantly lower Zn uptake than those that received field inoculum. The results indicate that mycorrhizal infection may have exerted some protective effect against plant Zn accumulation at the range of soil Zn concentrations studied and may have immobilised Zn in or near the roots to some extent. However, this mycorrhizal effect cannot be explained simply by tissue dilution, hyphal sequestration or root immobilisation of Zn. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Arbuscular mycorrhiza; Inoculum type; Soil pollution; White clover; Zinc

1. Introduction

The principal effect of arbuscular mycorrhiza in agricultural production systems and natural ecosystems is in most cases the capability to supply the host plant with mineral nutrients that are relatively immobile in the soil, particularly phosphorus and trace elements (Li et al., 1991; Jakobsen et al., 1994). In a glasshouse experiment, Thompson (1996) demonstrated that arbuscular mycorrhizal inoculation could correct dual phosphorus and zinc deficiencies of linseed plants. However, elevated concentrations of heavy metals exist in many agricultural soils from anthropogenic sources

such as sewage sludge application (McGrath, 1987) or mining activities (Shetty et al., 1995; Weissenhorn et al., 1995) and this poses long-term risks to environmental quality and sustainable food production. It has been widely reported that ectomycorrhizal and ericoid mycorrhizal fungi can increase the tolerance of their host plants to heavy metals when these are present at toxic levels (Bradley et al., 1981, 1982; Jones and Hutchinson, 1988a,b). The underlying mechanism of the enhancement of plant tolerance to heavy metal contamination is thought to be the binding capacity of fungal hyphae to metals in the roots or in the rhizosphere. This immobilises the metals in or near the roots and thus decreases their translocation to the shoots (Bradley et al., 1981; Brown and Wilkins, 1985; Wasserman et al., 1987). As far as the role of arbuscular mycorrhiza in plant heavy metal uptake is concerned, no firm general conclusions have been drawn.

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In some reports it has been claimed that arbuscular mycorrhiza may also help plants to tolerate high levels of soil heavy metal contamination. For example, Heggio et al. (1990) and Hetrick et al. (1994) were able to demonstrate that at high soil heavy metal concentrations, arbuscular mycorrhizal infection reduced the concentrations of Zn, Cd and Mn in plant leaves. However, there has been no published evidence showing the quantitative relationship between mycorrhizal infection and plant tolerance to heavy metal pollution under controlled conditions. The present study was therefore, designed to investigate the effects of different levels of soil Zn contamination on the infection of white clover by indigenous arbuscular mycorrhizal in glasshouse conditions and the relationship between mycorrhizal infection and plant heavy metal tolerance.

2. Materials and methods

2.1. Soil preparation

Soil was collected from a mixed perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) sward in a long-term field experiment at the Agricultural Research Institute of Northern Ireland at Hillsborough, County Down (Irish Grid Reference J244577). The soil was a sandy clay loam derived from Silurian shale and is typical of a large area of County Down. Moist samples removed from the top 10 cm of the soil profile were passed through a 0.5-cm sieve, and large stones and plant root debris were removed. The soil was then thoroughly mixed and sterilised with 10 kGy of γ -irradiation using a ^{60}Co source. The soil contained 50 mg 'total' (aqua-regia – extractable) Zn kg⁻¹ dry soil. Irradiated soil was mixed with sterile (autoclaved) acid-washed sand in a 50:50 w/w ratio to aid drainage and aeration. Aliquots of the soil/sand mixture were amended with five levels of Zn addition: 0, 50, 100, 200 and 400 mg kg⁻¹ soil as ZnSO₄ solution. K₂SO₄ solution was also applied at the appropriate rates to balance the quantity of S added to each treatment and thus prevent plant yield responses to S.

2.2. Mycorrhizal inocula

Two types of inoculum were compared. Field inoculum comprised fresh roots collected from the same field from which the soil was obtained. The roots were derived from areas dominated by white clover, but some roots of other species, especially perennial ryegrass, would have been present. The second type of inoculum consisted of fresh roots of white clover from trap culture using the same clover cultivar as in the pot experiment.

2.3. Glasshouse experiment

Four three-week old white clover seedlings (*T. repens* cv. Grasslands Huia) were transferred to each 9-cm plastic plant pot containing 300 g of soil/sand mixture. A mycorrhiza inoculum pad (0.3 g of fresh roots) was placed immediately around the root systems. 10 ml of a suspension of *Rhizobium leguminosarum* biovar. *trifolii* were added to each pot to ensure effective nodulation of the roots. The experiment consisted of five levels of Zn addition (0, 50, 100, 200 and 400 mg kg⁻¹) and three mycorrhizal treatments (no inoculum, field inoculum and trap culture inoculum), giving 15 treatments with ten replicates in a fully randomised block. The pots were adjusted regularly to field capacity using tap water. Mercury vapour lamps provided an additional 150–400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation (PAR) at pot level from 8 to 16 each day. Supplementary heating was used to maintain a minimum temperature of 10°C. The plants were harvested after 20 weeks. The shoots were removed by cutting just above the soil surface. The roots were recovered by washing with de-ionised water. Roots and shoots were weighed after oven drying at 70°C overnight and then ground to <0.25 mm.

2.4. Chemical analysis of plant material

Subsamples of ground oven dried roots and shoots were digested in a mixture of nitric and perchloric acids to destroy the organic matter (Ministry of Agriculture, Fisheries and Food, 1986). Multi-element analysis, including P and Zn, was then performed using inductively coupled plasma-atomic emission spectroscopy (ICP-AES).

2.5. Determination of mycorrhiza infection

Subsamples of washed plant roots were gently dried with tissue paper and preserved in formalin–glacial acetic acid–ethanol (12.5:12.5:200 v/v). Prior to examination, roots were cut into 2-cm bundles and rinsed under tap water. Randomly selected root segments were then cleared in 5% v/v KOH for 60 min at 90°C, followed by thorough washing with water. The cleared root segments were soaked overnight in 1% v/v HCl at room temperature and placed in the stain solution (0.05% w/v Trypan blue in lactoglycerol) for 15 min at 60°C. Stained root segments were mounted on microscope slides under coverslips. A magnified intersection method (McGonigle et al., 1990) was used to determine the proportion of root length infected. Five stained root segments were aligned perpendicular to the long axis of each slide and observed at $\times 200$ magnification. The field of view was moved systematically without looking through the microscope so that 50 intersections between

roots and a vertical eyepiece cross hair were counted as either negative or positive (i.e. with arbuscules, vesicles or hyphae) per slide. Three slides were examined for each treatment, giving a total of 150 counts per sample as recommended by McGonigle et al. (1990).

3. Results

3.1. Mycorrhiza infection

No infection was observed in the uninoculated control plants. Table 1 shows that the mean proportion of root length infected in inoculated plants ranged from 33% to 46% and there was no significant difference between field and trap culture inocula. There seemed to be a slight trend of increasing infection with increasing addition of Zn to the soil, but the only treatment that showed a significant difference from the unamended control was the highest application rate of Zn combined with trap culture inoculum. Thus, in general, there was little effect of Zn application rate on mycorrhiza infection rate, and there was certainly no depression of infection resulting from the moderate rates of Zn contamination of the soil studied.

3.2. Root and shoot yields

Mycorrhizal plants treated with trap culture inoculum had lower shoot yields than non-mycorrhizal controls, and mycorrhizal plants treated with field inoculum had higher root yields than non-mycorrhizal controls. Although these yield effects were statistically significant (Table 2), they were small. There was no consistent trend between Zn application rate and shoot yield, but at the highest Zn application rate (400 mg kg⁻¹) there tended to be a decrease in shoot yield (not significant for trap culture inoculum). Application of Zn had little effect on root yield.

3.3. Shoot and root P concentrations

Mycorrhizal plants had higher shoot and root P concentrations than non-mycorrhizal controls (Table 3). Mycorrhizal plants that received trap culture inoculum had higher root and shoot P concentrations than those treated with field inoculum. The highest Zn application rate gave higher shoot and root P concentrations than did any other application rate.

3.4. Root and shoot Zn concentrations

The highest Zn shoot concentrations were found in the non-mycorrhizal control plants, with significantly lower concentrations in those treated with field inoculum and the lowest concentrations in plants that received trap culture inoculum (Table 4). Root Zn concentrations were also significantly higher in non-mycorrhizal than in mycorrhizal plants, but there was no effect of inoculum type on root Zn concentration. There were clear increases in shoot and root Zn concentrations with increasing Zn application rate to the soil. Zinc concentrations were higher in the roots than in the shoots.

3.5. Root and shoot Zn uptake

Table 5 shows that the plant Zn uptake results broadly reflect the root and shoot Zn concentrations because of the small effects of infection and Zn application on root and shoot yields. However, marked differences between inoculum types are now evident in both roots and shoots, with lower Zn uptake occurring in both roots and shoots of plants receiving trap culture than in those treated with field inoculum. Shoot and root Zn uptakes were similar when no Zn was applied but as Zn application rate increased, shoot Zn uptake increased much less than root Zn uptake and the decrease in Zn uptake by mycorrhizal roots and shoots compared with controls became more pronounced.

Table 1
Mycorrhiza infection rates of white clover roots (percentage of total root length infected)

Inoculum type	Added Zn (mg kg ⁻¹ soil/sand)				
	0	50	100	200	400
Field inoculum	40.9	33.9	39.1	40.0	46.1
Trap culture inoculum	36.0	32.9	38.4	37.8	49.6
Standard error			3.62		
Significance ^a due to:					
Inoculum type			NS		
Zn application rate			**		
Inoculum type × Zn rate			NS		

^a By analysis of variance; **, $P < 0.01$; NS, not significant.

Table 2

Effect of mycorrhizal infection and added Zn on white clover shoot and root yield (g DM pot⁻¹)

Inoculum type	Added Zn (mg kg ⁻¹ soil/sand)				
	0	50	100	200	400
Shoots					
No inoculum (control)	3.98	3.63	3.83	3.86	3.53
Field inoculum	3.78	3.83	3.89	3.75	3.21
Trap culture inoculum	3.51	3.77	3.60	3.68	3.30
Standard error			0.121		
Significance ^a of differences due to:					
Inoculum type			*		
Zn application rate			***		
Inoculum type × Zn rate			NS		
Roots					
No inoculum (control)	1.33	1.19	1.36	1.31	1.29
Field inoculum	1.48	1.41	1.44	1.51	1.39
Trap culture inoculum	1.33	1.24	1.32	1.38	1.30
Standard error			0.051		
Significance ^a of differences due to:					
Inoculum type			***		
Zn application rate			*		
Inoculum type × Zn rate			NS		

^a By analysis of variance; *, $P < 0.05$; ***, $P < 0.001$.

Table 3

Effect of mycorrhizal infection and added Zn on white clover shoot and root P concentration (% DM)

Inoculum type	Added Zn (mg kg ⁻¹ soil/sand)				
	0	50	100	200	400
Shoots					
No inoculum (control)	0.094	0.092	0.100	0.097	0.112
Field inoculum	0.146	0.142	0.156	0.155	0.180
Trap culture inoculum	0.165	0.153	0.170	0.163	0.198
Standard error			0.0053		
Significance of differences due to:					
Inoculum type			***		
Zn application rate			*		
Inoculum type × Zn rate			NS		
Roots					
No inoculum (control)	0.108	0.109	0.109	0.108	0.130
Field inoculum	0.198	0.189	0.205	0.198	0.227
Trap culture inoculum	0.213	0.197	0.217	0.195	0.232
Standard error			0.0052		
Significance of differences due to:					
Inoculum type			***		
Zn application rate			***		
Inoculum type × Zn rate			NS		

4. Discussion

Our results are in broad agreement with those of Heggo et al. (1990), who found that mycorrhizal infec-

tion of soybean plants increased foliar P uptake and decreased Zn uptake in highly polluted soils. They also found increases in foliar P concentration and decreases in foliar Zn concentration in soils with higher Zn con-

Table 4

Effect of mycorrhizal infection and added Zn on clover shoot and root Zn concentration (mg kg⁻¹ DM)

Inoculum type	Added Zn (mg kg ⁻¹ soil/sand)				
	0	50	100	200	400
Shoots					
No inoculum (control)	22.7	67.7	100.5	126.0	235.9
Field inoculum	25.9	40.6	51.8	83.9	167.3
Trap culture inoculum	19.3	41.3	49.0	65.2	116.0
Standard error			3.85		
Significance of differences due to:					
Inoculum type			***		
Zn application rate			***		
Inoculum type × Zn rate			***		
Roots					
No inoculum (control)	69.5	257.4	402.0	605.5	1123.4
Field inoculum	53.6	120.5	178.0	273.1	637.2
Trap culture inoculum	61.9	106.2	170.9	243.1	538.7
Standard error			35.47		
Significance of differences due to:					
Inoculum type			***		
Zn application rate			***		
Inoculum type × Zn rate			***		

Table 5

Effect of mycorrhizal infection and added Zn on white clover shoot and root Zn uptake (μg pot⁻¹)

Inoculum type	Added Zn (mg kg ⁻¹ soil/sand)				
	0	50	100	200	400
Shoots					
No inoculum (control)	91	245	381	485	836
Field inoculum	98	155	203	315	540
Trap culture inoculum	68	156	177	240	382
Standard error					
Significance of differences due to:					
Inoculum type			***		
Zn application rate			***		
Inoculum type × Zn rate			***		
Roots					
No inoculum (control)	92	311	549	800	1445
Field inoculum	79	168	255	408	888
Trap culture inoculum	82	130	222	339	697
Standard error					
Significance of differences due to:					
Inoculum type			***		
Zn application rate			***		
Inoculum type × Zn rate			***		

tamination from mining activities, and the effect of arbuscular mycorrhiza was related to initial soil metal concentration. The absence of any effect of Zn application on mycorrhizal infection rate in our experiment

may be attributable to the application of inoculum immediately around the roots of the seedlings. This may have promoted root colonisation at an early stage of plant growth.

According to Smith and Read (1997), a number of different mechanisms may be involved in the interactions between mycorrhizal colonisation and accumulation of heavy metals, including tissue dilution of the toxic element due to interactions with P nutrition (and increased yield), sequestration of the toxic metal in the fungus and development of tolerance by the fungus. In our experiment, tissue dilution is unlikely to have been important because mycorrhizal infection had a relatively small effect on plant yield. The soil Olsen P concentration was 32 mg l^{-1} at the start of the experiment. This would not be considered to be a low available P status and would not be expected to show P deficiency in non-mycorrhizal or unfertilised treatments, and thus would be unlikely to show a strong yield response to mycorrhizal inoculation. Although there was significantly higher Zn uptake by roots than by shoots of mycorrhizal white clover plants, both root and shoot uptake were much lower than in non-mycorrhizal control plants in Zn contaminated soil. Therefore, sequestration by the fungal mycelium cannot account solely for the lower shoot uptake of the metal. Our root samples would have included internal fungal tissue and perhaps some adhering external mycelium. It could be argued that Zn was sequestered in the bulk external mycelium which was not included in the root samples, but it seems unlikely that this could account for the very marked decrease in root Zn uptake in inoculated plants. Recent work on *Medicago tunculata* by Burleigh (personal communication) has indicated that arbuscular mycorrhiza down-regulates a putative low-affinity Zn transporter, thus the lower uptake by mycorrhizal clover plants in contaminated soil may be due to the suppression of a gene that controls uptake from high external Zn concentrations.

It is unlikely that development of tolerance to Zn by the fungi could explain the results in our short-term experiment. However, trap culture inoculum conferred significantly greater protection to the plants than field inoculum, and this may be due to the development of a more effective suite of fungal species or strains during the growth of the clover plants in the trap cultures.

In summary, we have demonstrated a protective effect of arbuscular mycorrhiza against uptake of potentially toxic Zn by white clover growing in moderately polluted soil. The level of protection was higher when inoculum was used consisting of white clover roots in which arbuscular mycorrhiza had developed in trap cultures, possibly leading to a more highly specialised relationship between the host and the fungi (from expression of ecological specificity) than with field inoculum (Zhu et al., 2000). It is interesting to note that arbuscular mycorrhiza seems to protect host plants by reducing uptake of toxic metal into roots and shoots, but hyperaccumulator plant species that have developed tolerance to very high soil metal concentrations show increased translocation of metal to the shoots (Brooks,

1998). Many hyperaccumulators belong to plant families that are often considered to be non-mycorrhizal (for example the Brassicaceae). Further research, including studies on mycorrhizal hyperaccumulator species (if possible), is required to elucidate the mechanism of mycorrhizal protection against toxic elements in soils.

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