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ORIGINAL PAPER

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Uptake of cadmium from an experimentally contaminated calcareous soil by arbuscular mycorrhizal maize (*Zea mays* L.)

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Abstract We investigated uptake of Cd by arbuscular mycorrhizal (AM) maize inoculated with *Glomus mosseae* from a low-P sandy calcareous soil in two glasshouse experiments. Plants grew in pots containing two compartments, one for root and hyphal growth and one for hyphal development only. Three levels of Cd (0, 25 and 100 mg kg^{-1}) and two of P (20 and 60 mg kg^{-1}) were applied separately to the two compartments to assess hyphal uptake of Cd. Neither Cd nor P addition inhibited root colonization by the AM fungus, but Cd depressed plant biomass. Mycorrhizal colonization, P addition and increasing added Cd level led to lower Cd partitioning to the shoots. Plant P uptake was enhanced by mycorrhizal colonization at all Cd levels studied. When Cd was added to the plant compartment and P to the hyphal compartment, plant biomass increased with AM colonization and the mycorrhizal effect was more pronounced with increasing Cd addition. When P was added to the plant compartment and Cd to the hyphal compartment, plant biomass was little affected by AM colonization, but shoot Cd uptake was increased by colonization at the low Cd addition rate (25 mg kg^{-1}) and lowered at the higher Cd rate (100 mg kg^{-1}) but with no difference in root Cd uptake. These effects may have been due to immobilization of Cd by the fungal mycelium or effects of the AM fungus on rhizosphere physicochemical conditions and are discussed in relation to possible phytostabilization of contaminated sites by AM plants.

Keywords Cadmium contamination · Phosphorus nutrition · Soil remediation · Phytostabilization · Arbuscular mycorrhiza

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Introduction

Cd is a potentially phytotoxic heavy metal (di Toppi and Gabbrielli 1999). It is released into the environment by various industrial and agricultural activities including mining, metal smelting and application of sewage sludge to agricultural land (Nriagu and Pacyna 1988; Chen et al. 1999), sometimes resulting in accumulation of the metal in soils. Excessive Cd levels represent a threat to soil productivity and ecosystem stability, and also to human and animal health due to Cd accumulation in food chains (Jackson and Alloway 1991). There is therefore considerable interest in management of Cd in plant-soil systems and in strategies for remediation of Cd-contaminated soils or improving plant tolerance to Cd contamination.

It is well known that arbuscular mycorrhizal (AM) fungi are widely distributed in natural and agricultural ecosystems (Harley 1989). Although the initial plant colonizers of heavy metal-contaminated sites are often species tolerant to extreme conditions and tend to be non-mycorrhizal (Shetty et al. 1994), they may increase soil organic matter content, modify the soil microclimate and promote conditions conducive to the establishment of species with stronger mycorrhizal dependence (Leyval et al. 1997). Mycorrhizas have recently been reported in plants growing on heavy metal-contaminated sites (Shetty et al. 1995; Weissenhorn and Leyval 1995; Pawlowska et al. 1996; Chaudhry et al. 1999), and metal-tolerant fungal strains have been isolated (Griffioen 1994; Weissenhorn et al. 1993, 1994; Hildebrandt et al. 1999). Alleviation of heavy metal phytotoxicity by arbuscular mycorrhiza has been indicated in several studies (Schüepp et al. 1987;

Heggo et al. 1990; Zhu et al. 2001; Chen et al. 2003). The AM fungi may act indirectly, for example by enhancing plant P nutrition and increasing plant growth with a resulting dilution effect of the metal in the host plant, or directly by binding of the metal to the fungal mycelium and immobilization in the rhizosphere or the roots (Galli et al. 1994; Joner et al. 2000; Chen et al. 2001). Non-mycorrhizal metal hyperaccumulators may thus be suitable for phytoremediation of contaminated sites while mycorrhizal colonizers may be more useful for phytostabilization of heavy metals in the field.

Using a compartmented pot system, Joner and Leyval (1997) demonstrated that ^{109}Cd added to the hyphal compartment was adsorbed by extraradical hyphae and subsequently transported to plant roots, but transfer from fungus to plants was restricted by fungal immobilization. Weissenhorn et al. (1993) demonstrated stronger tolerance to Cd in AM fungal strains isolated from heavy metal-polluted soils. The potential use of AM fungi in remediation programs for metal-contaminated ecosystems has recently been proposed (Vangronsveld et al. 1996; Khan et al. 2000; Leyval et al. 2002). However, more information is required on the mechanisms involved in mycorrhiza-mediated uptake of Cd by plants in contaminated soils and the effects of soil factors such as P supply.

This paper describes two glasshouse experiments carried out to investigate the mycorrhizal effects on plant P and Cd uptake. Plexiglas pots were separated with nylon net of 30-μm mesh size into two compartments for plant growth and hyphal development. Additions of Cd and P were made differentially to the two compartments to avoid direct interactions between the two elements. In the first experiment Cd was added to the plant compartment at three rates (0, 25 or 100 mg Cd kg soil), and two levels of P (20 or 60 mg P kg^{-1} soil) were added to the $hyphal^{-1}$ compartment, while in the second experiment Cd was added to the hyphal compartment, and P to the plant compartment to discriminate between direct root uptake and indirect uptake of P and Cd via the external mycelium of the AM fungus.

Materials and methods

Mycorrhizal inoculum

The AM fungus *Glomus mosseae* (Nicol & Gerd) Gerdemann & Trappe was kindly provided by Professor H. G. Wang of the Institute of Plant Nutrition and Fertilizers, Chinese Academy of Agricultural Sciences. The fungus was propagated in pot culture on maize plants grown in a sandy soil for 10 weeks. Inoculum from pot culture was a mixture of spores, mycelium, sandy soil and maize root fragments containing approximately 1,000 spores per 100 g.

Host plants

Seeds of maize (*Zea mays* L. cv. ND108) were surface sterilized in a 10% (v/v) solution of hydrogen peroxide for 10 min then immersed in deionised water for 6 h. They were pre-germinated on moist filter paper overnight at 25°C. The pre-germinated seeds were selected for uniformity of size before sowing.

Calcareous soil

A calcareous sandy soil of low nutrient status was collected from the vicinity of Lugouqiao, Fengtai district, Beijing. The soil had the following properties (dry matter basis): pH (soil:water ratio 1:2.5) 8.67, 0.30% organic matter, 0.018% Kjeldahl-N, 3.4 mg kg^{-1} of 0.5 mol l^{-1} $NaHCO_3$-extractable P (Olsen et al. 1954; Murphy and Riley 1962), and 33.6 mg kg^{-1} of 1 mol l^{-1} NH_4OAc-exchangeable K. Soil-extractable trace element concentrations were: 4.96 mg kg^{-1} Fe, 4.15 mg kg^{-1} Mn, 0.28 mg kg^{-1} Cu, 0.48 mg kg^{-1} Zn, and 0.06 mg kg^{-1} Cd. Soil metals were extracted with a 5 mmol l^{-1} diethylene triamine pentaacetic acid-10 mmol l^{-1} $CaCl_2$–0.1 mol l^{-1} triethanolamine solution adjusted to pH 7.30 (Lindsay and Norvell 1978) and metal concentrations were determined using an atomic absorption spectrophotometer (Perkin Elmer Model 2100). Soil was passed through a 1-mm sieve, sterilized by autoclaving at 120°C for 2 h and then air-dried.

Experimental procedure

Plexiglas pots had two compartments of equal volume separated by nylon net of 30-μm mesh size to allow penetration by hyphae but not by roots. One compartment was for plant (and hyphal) growth and the other for hyphal growth only. The pots were rectangular boxes 15 cm high, 10 cm deep and 10 cm wide.

In both experiments there were three Cd addition levels (0, 25 or 100 mg Cd kg^{-1} soil as $CdSO_4$ solution) and two P addition levels (20 and 60 mg P kg^{-1} soil as KH_2PO_4 solution). Maize plants were inoculated with the AM fungus *G. mosseae* at each Cd and P addition rate and uninoculated controls were included. Thus, there were 12 treatments with four replicates giving a total of 48 pots in each experiment. All treatments received mineral nutrients applied in solution to each compartment at rates of 300 mg N (NH_4NO_3), 20 mg P (KH_2PO_4: only to compartments with no P addition treatments) and 200 mg K (K_2SO_4) kg^{-1} soil as a basal application.

Each plant compartment contained 800 g soil and the hyphal compartment contained 900 g soil. Inoculated treatments received 40 g of inoculum, while uninoculated plants received the same weight of sterilized inoculum together with a filtrate (0.25 μm pore size) of unsterilized soil to provide a similar microflora except for the absence of the mycorrhizal fungus. The inoculum was thoroughly mixed with the 800 g of fertilized soil and the soil was then placed in the plant compartment. Four selected pre-germinated maize seeds were sown in the plant compartment of each pot and thinned to two plants 7 days after seedling emergence. Soil moisture content was adjusted regularly to ~70% water holding capacity with deionized water during the course of the experiments.

The experiments were conducted in the glasshouse of the Department of Plant Nutrition, China Agricultural University, and the plants grew for 7 weeks from 22 April 2001 to 9 June 2001 without supplementary illumination under a daytime temperature range of 20–30°C and a night temperature range of 15–20°C.

Harvest and chemical analysis

Shoots and roots were harvested separately. Samples were carefully washed with tap water and then deionised water. Sub-samples of fresh roots were collected for determination of root colonization rate by the AM fungus. The dry weights of leaves, stems and roots were determined after oven drying at 70°C for 48 h. Oven-dried sub-samples were milled to pass through a 0.5-mm sieve and dry ashed at 560°C for multi-element analysis by inductively coupled plasma-atomic emission spectroscopy (ICP-AES; Perkin Elmer Optima 3300DV) (Sahrawat et al. 2002; Sun et al. 2000).

Soil pH was measured with a pH meter using a soil:water ratio of 1:2.5. Root samples for assessing root colonization were

prepared using the acid fuchsin staining-grid intersect method (Kormanik et al. 1979; Giovannetti and Mosse 1980).

Statistical analysis

Root colonization data of mycorrhizal treatments were tested by two-way ANOVA to compare P and Cd levels, and other variables by three-way ANOVA to compare mycorrhizal status and P and Cd levels using GenStat for PC/Windows Release 6.1 (Payne 2002).

Results

Experiment 1

Mycorrhizal colonization

No root infection was detected in uninoculated plants but roots of inoculated plants were extensively mycorrhizal, with the mean proportion of root length colonized ranging from 55 to 71% (Table 1). There was little effect of Cd addition level to the plant compartment on root colonization rate at the lower P level in the hyphal compartment. At the higher P level, root colonization was depressed to 55% at the lower Cd level (25 mg kg^{-1}) compared with controls receiving no Cd and increased to 71% at the higher Cd addition level of 100 mg kg^{-1}. P addition to the hyphal compartment had no effect on mycorrhizal colonization rate (Table 1).

Shoot and root biomass

Mycorrhizal colonization led to significantly higher shoot biomass ($P<0.001$) compared with non-mycorrhizal maize but had no effect on root biomass (Table 1). The P level in the hyphal compartment had no effect on shoot or root biomass, but a small interaction in root biomass occurred between colonization and P level ($P<0.05$) because root biomass tended to be higher in mycorrhizal plants at the lower P level and the opposite trend occurred at the higher P level. Both shoot and root biomass decreased with increasing rate of Cd application to the plant compartment.

When no Cd was added to the plant compartment, mycorrhizal colonization led to higher shoot biomass but had no influence on root biomass. When 25 mg Cd kg^{-1} was added to the plant compartment and 20 mg P kg^{-1} to the hyphal compartment, both shoot and root yields

Table 1 Mean root colonization (percentage of root length colonized by *Glomus mosseae*), shoot and root biomass [g dry matter (DM) pot^{-1}], shoot P and Cd uptake (mg pot^{-1}) and ratio of shoot Cd uptake:root Cd uptake (*S:R Cd uptake*) of maize plants in experiment 1 with three Cd levels [0 mg Cd kg^{-1} (*Cd 0*), 25 mg Cd kg^{-1} (*Cd 25*) and 100 mg Cd kg^{-1} (*Cd 100*)] added to the plant compartment and two levels of P [20 mg P kg^{-1} (*P20*) and 60 mg P kg^{-1} (*P60*)] added to the hyphal compartment of mycorrhizal [inoculated with the arbuscular mycorrhizal (AM) fungus *G. mosseae*] and non-mycorrhizal plants. Significance of *F*-ratios by two-way ANOVA for root colonization of mycorrhizal plants and by three-way ANOVA for other variables. *n.d.* Not determined (because Cd concentrations in plants receiving no added Cd were below the detection limit)

	P20			P60		
Variable	Cd 0	Cd 25	Cd 100	Cd 0	Cd 25	Cd 100
Root colonization						
Mycorrhizal	63.6	60.9	61.5	64.6	54.6	71.4
Significance	P level n.s., Cd level *, P×Cd n.s.					
Shoot biomass						
Mycorrhizal	6.61	6.76	5.32	6.53	5.82	5.17
Non-mycorrhizal	6.08	4.77	4.08	6.00	5.66	4.09
Significance	Mycorrhiza***, P level n.s., Cd level***, Mycorrhiza×P n.s., Mycorrhiza×Cd n.s., P×Cd n.s.					
Root biomass						
Mycorrhizal	4.78	4.50	2.94	4.29	3.90	3.00
Non-mycorrhizal	4.07	3.13	3.15	4.54	4.34	3.19
Significance	Mycorrhiza n.s., P level n.s., Cd level***, Mycorrhiza×P*, Mycorrhiza×Cd n.s., P×Cd n.s					
Shoot P uptake						
Mycorrhizal	11.1	12.8	12.0	12.5	13.5	12.8
Non-mycorrhizal	3.9	3.2	3.2	3.6	3.9	2.7
Significance	Mycorrhiza***, P level n.s., Cd level n.s., Mycorrhiza×P n.s., Mycorrhiza×Cd n.s., P×Cd n.s.					
Shoot Cd uptake						
Mycorrhizal	n.d.	0.094	0.221	n.d.	0.138	0.196
Non-mycorrhizal	n.d.	0.126	0.198	n.d.	0.164	0.223
Significance	Mycorrhiza*, P level**, Cd level***, Mycorrhiza×P n.s., Mycorrhiza×Cd n.s., P×Cd**					
S:R Cd uptake						
Mycorrhizal	n.d.	0.148	0.125	n.d.	0.167	0.122
Non-mycorrhizal	n.d.	0.252	0.130	n.d.	0.231	0.142
Significance	Mycorrhiza **, P level n.s., Cd level ***, Mycorrhiza×P n.s., Mycorrhiza×Cd *, P×Cd n.s.					

*** $P<0.001$, ** $P<0.01$, * $P<0.05$, *n.s.* not significant

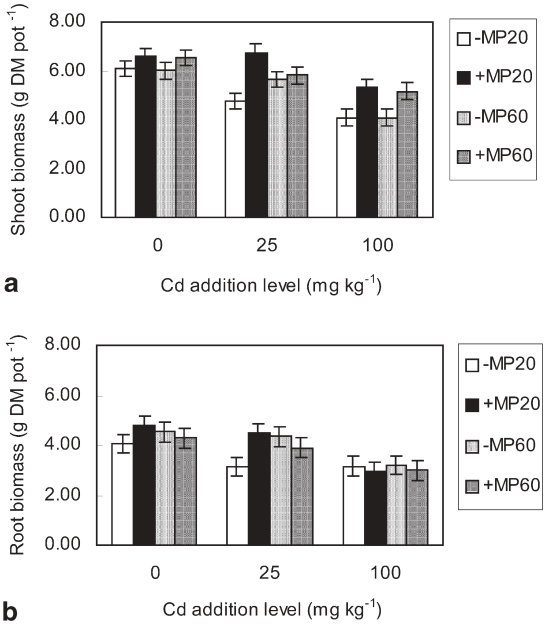

Fig. 1 Shoot (**a**) and root (**b**) biomass of maize plants in experiment 1 with three Cd levels added to the plant compartment and two levels of P [20 mg P kg^{-1} (*P20*) and 60 mg P kg^{-1} (*P60*)] added to the hyphal compartment of non-mycorrhizal (*–M*) and mycorrhizal [*+M;* inoculated with the arbuscular mycorrhizal (AM) fungus *G. mosseae*] plants. *Bars* indicate ±1 SEM as estimated from three-way ANOVA

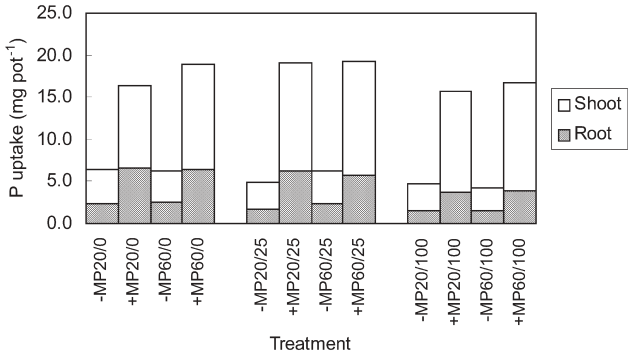

Fig. 2 Shoot and root P uptake of maize plants in experiment 1 with three Cd levels (0, 25 and 100 mg Cd kg^{-1}) added to the plant compartment and two P levels (P20 and P60) added to the hyphal compartment of –M and +M (inoculated with the AM fungus *G. mosseae*) plants. SEMs as estimated from three-way ANOVA were 0.70 and 0.44 mg P pot^{-1} for shoots and roots, respectively. For abbreviations, see Fig. 1

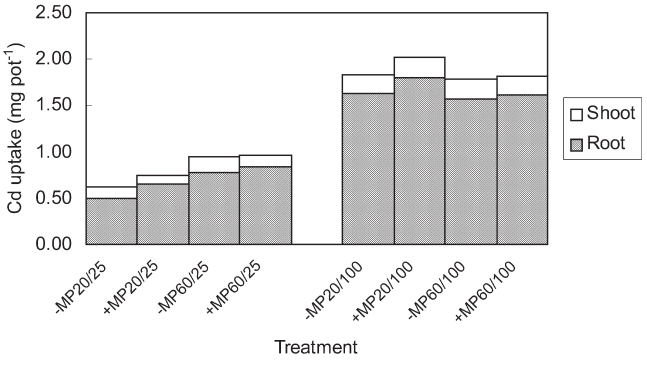

Fig. 3 Shoot and root Cd uptake by maize plants in experiment 1 with two levels of Cd (25 and 100 mg Cd kg^{-1}) added to the plant compartment and two levels of P (P20 and P60) added to the hyphal compartment of –M and +M (inoculated with the AM fungus *G. mosseae*) plants. SEMs as estimated from three-way ANOVA were 0.010 and 0.107 mg Cd pot^{-1} for shoots and roots, respectively. For abbreviations, see Fig. 1

increased with mycorrhizal colonization, while plant growth was unaffected at the P addition rate of 60 mg kg^{-1}. At the Cd addition level of 100 mg kg^{-1} in the plant compartment, shoot biomass was substantially increased but root biomass decreased with mycorrhizal colonization and there was no effect of P addition treatment (Fig. 1).

Maize plants exhibited severe symptoms of Cd toxicity with chlorosis and withering of old leaves and stunting of the plants, especially at the higher Cd addition level of 100 mg kg^{-1}, and these symptoms appeared to be alleviated by mycorrhizal colonization. Typical P deficiency symptoms were also observed in plants from all uninoculated treatments, but not in any of the mycorrhizal plants.

Shoot P uptake

Shoot P uptake was significantly higher with mycorrhizal colonization ($P<0.001$), but was unaffected by P addition treatment irrespective of Cd addition level (Table 1). Root P uptake decreased with the increasing Cd addition level but shoot P uptake was unaffected (Fig. 2).

Shoot Cd uptake

Shoot Cd uptake increased greatly with increasing Cd addition levels to the plant compartment ($P<0.001$). Shoot Cd uptake was generally higher than root uptake in the controls receiving no added Cd. Plants receiving 25 mg kg^{-1} added Cd showed lower shoot Cd uptake but higher root Cd uptake with mycorrhizal colonization. P addition to the hyphal compartment generally resulted in higher plant Cd uptake. At the Cd addition level of 100 mg kg^{-1}, neither P nor Cd addition showed any discernible influence on plant Cd uptake (Fig. 3).

Relatively less Cd was partitioned to the shoots at higher Cd addition levels as indicated by the lower shoot:root ratio of Cd uptake under the 100 mg kg^{-1} Cd addition level compared with 25 mg kg^{-1} of added Cd and the zero-Cd controls. Mycorrhizal colonization was also associated with lower Cd partitioning to the shoots, while

Table 2 Mean root colonization (percentage of root length colonized by *G. mosseae*), shoot and root biomass (g DM pot^{-1}), shoot P and Cd uptake (mg pot^{-1}) and S:R Cd uptake of maize plants in experiment 2 with three Cd levels (0 Cd, 25 Cd and 100 Cd) added to the hyphal compartment and two levels of P (P20 and P60) added to the plant compartment of mycorrhizal (inoculated with the AM fungus *G. mosseae*) and non-mycorrhizal plants. Significance of *F*-ratios by two-way ANOVA for root colonization of mycorrhizal plants and by three-way ANOVA for other variables. For abbreviations, see Table 1

	P20			P60		
Variable	Cd 0	Cd 25	Cd 100	Cd 0	Cd 25	Cd 100
Root colonization						
Mycorrhizal	59.7	52.6	53.3	56.1	57.2	55.8
Significance	P level n.s., Cd level n.s., P×Cd n.s.					
Shoot biomass						
Mycorrhizal	7.12	6.68	5.60	7.30	6.73	5.80
Non-mycorrhizal	6.54	6.02	5.44	7.95	7.31	6.86
Significance	Mycorrhiza n.s., P level ***, Cd level ***, Mycorrhiza×P **, Mycorrhiza×Cd n.s., P×Cd n.s.					
Root biomass						
Mycorrhizal	4.25	4.54	3.66	3.69	3.60	3.80
Non-mycorrhizal	5.30	3.67	3.70	4.46	4.25	4.69
Significance	Mycorrhiza *, P level n.s., Cd level *, Mycorrhiza×P *, Mycorrhiza×Cd *, P×Cd **					
Shoot P uptake						
Mycorrhizal	9.9	10.6	5.6	15.0	12.6	8.0
Non-mycorrhizal	3.9	3.7	3.5	8.1	6.6	6.8
Significance	Mycorrhiza ***, P level ***, Cd level ***, Mycorrhiza×P n.s., Mycorrhiza×Cd ***, P×Cd n.s.					
Shoot Cd uptake						
Mycorrhizal	n.d.	0.007	0.012	n.d.	0.007	0.009
Non-mycorrhizal	n.d.	0.005	0.014	n.d.	0.006	0.014
Significance	Mycorrhiza n.s., P level n.s., Cd level ***, Mycorrhiza×P n.s., Mycorrhiza×Cd ***, P×Cd n.s.					
S:R Cd uptake						
Mycorrhizal	n.d.	0.112	0.079	n.d.	0.126	0.043
Non-mycorrhizal	n.d.	0.125	0.129	n.d.	0.118	0.061
Significance	Mycorrhiza n.s., P level *, Cd level ***, Mycorrhiza×P n.s., Mycorrhiza×Cd n.s., P×Cd *					

***$P<0.001$, **$P<0.01$, *$P<0.05$, *n.s.* not significant

P addition had no marked effect on Cd partitioning in the plants (Tables 1, 2).

Experiment 2

Mycorrhizal colonization

As in experiment 1, no root infection was detected in uninoculated plants while the proportion of root length of inoculated plants colonized ranged from 53 to 60% (Table 2), and mycorrhizal colonization was unaffected by Cd or P addition treatments.

Shoot and root biomass

The plants appeared healthy compared to those in experiment 1 with no severe symptoms of chlorosis in any treatment. Shoot biomass was markedly decreased by Cd addition to the hyphal compartment but there was no significant difference in root biomass between the two Cd addition levels (Table 2). Under all Cd addition levels, P additions to the plant compartment increased the biomass of uninoculated but not of inoculated plants (Fig. 4). When no Cd was added to the hyphal compartment, P

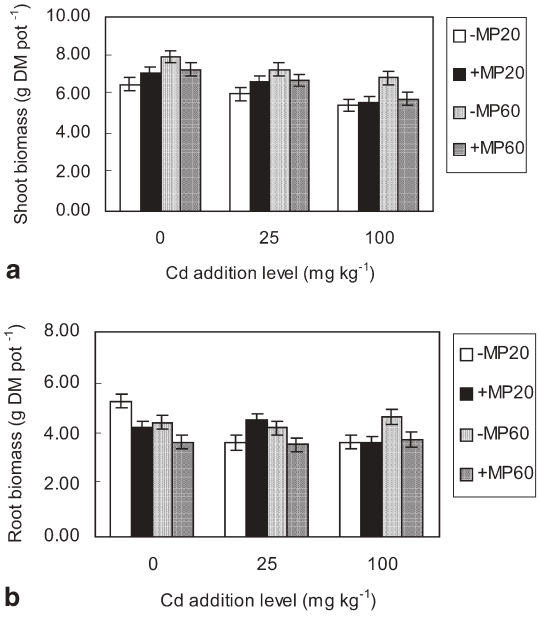

Fig. 4 Shoot (**a**) and root (**b**) biomass of maize plants in experiment 2 with three Cd levels added to the hyphal compartment and two levels of P (P20 and P60) added to the plant compartment of −M and +M (inoculated with the AM fungus *G. mosseae*) plants. *Bars* indicate ±1 SEM as estimated from three-way ANOVA

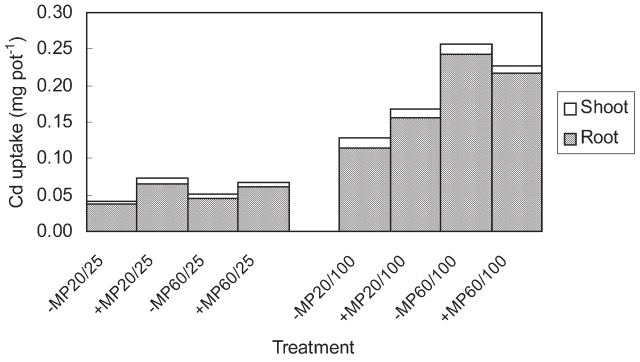

Fig. 5 Shoot and root Cd uptake by maize plants in Experiment 2 with two levels of Cd (25 and 100 mg Cd kg^{-1}) added to the hyphal compartment and two levels of P (P20 and P60:20 and 60 mg P kg^{-1}) added to the plant compartment of non-mycorrhizal (–M) and mycorrhizal (+M: inoculated with the AM fungus *G. mosseae*) plants. SEMs as estimated from three-way ANOVA were 0.001 and 0.009 mg Cd pot^{-1} for shoots and roots, respectively. For abbreviations, see Fig. 1

additions to the plant compartment depressed root biomass, while under Cd additions uninoculated plants had a larger root biomass at the higher P addition rate, with no such trends observed in inoculated plants (Fig. 4).

In general, shoot biomass was not significantly affected by mycorrhizal colonization, but at the P addition level of 20 mg kg^{-1} shoot biomass tended to increase with AM inoculation, with the opposite trend at the P addition level of 60 mg kg^{-1} (Fig. 4). On the other hand, mycorrhizal colonization led to a small but significant ($P<0.05$) depression in root growth (Table 2).

Shoot Cd uptake and hyphal contribution

When no Cd was added to the hyphal compartment only traces of Cd occurred in the plants. Under the Cd addition level of 25 mg kg^{-1}, Cd uptake by both shoots and roots was increased by mycorrhizal colonization. P addition showed no discernible influence on plant Cd uptake (Fig. 5). Under the highest Cd addition level of 100 mg kg^{-1}, shoot Cd uptake was lowered by mycorrhizal colonization, while root Cd uptake was increased by mycorrhizal colonization at the P addition rate of 20 mg kg^{-1} in the plant compartment, but with the elevated P addition rate of 60 mg kg^{-1} Cd uptake by both shoots and roots decreased due to mycorrhizal colonization (Fig. 5).

Discussion

High concentrations of Cd in the soil have been reported to inhibit mycorrhizal colonization (Gildon and Tinker 1983; Weissenhorn and Leyval 1995). Inhibitory effects of P fertilizer or high available P on AM fungal growth have also been found (e.g. Nagahashi et al. 1996). However, in the present study neither Cd nor P addition showed any negative effect on root colonization by *G. mosseae*. Relatively high root colonization rates occurred even at the highest Cd addition level. One possible explanation is that the plant roots were confined to a small volume of soil, so that any inhibitory effects of Cd or P on mycorrhizal colonization may have been minimized. In addition, it is possible that even the higher addition rate of P (60 mg kg^{-1}) did not fully ameliorate the available P deficiency in the calcareous soil used.

The results of the first experiment indicate that positive effects of mycorrhizal colonization on plant biomass were more pronounced under conditions of Cd contamination. In contrast, in the second experiment mycorrhizal colonization showed no significant influence on plant biomass. At the higher P addition level of 60 mg kg^{-1}, plant biomass was depressed by AM colonization. On the other hand, biomass of uninoculated plants increased with P addition to the plant compartment. The different plant biomass results may be ascribed to the maize plants experiencing "direct" Cd contamination in the first experiment in contrast to the second experiment in which only a small fraction of the added Cd was transferred to the plants via hyphal uptake, with a correspondingly small influence on plant biomass. In addition, relatively large amounts of carbohydrates in the plants may have been allocated to establishment of the mycorrhizal association and associated fungal growth (Harris and Paul 1987; Peng et al. 1993), and this may have masked the mycorrhizal contribution to P uptake.

Numerous studies have indicated that positive effects of mycorrhizas on host plants under conditions of environmental stress might be attributable, directly or indirectly, to enhanced plant P nutrition. However, in our second experiment plant P uptake (data not shown), but not plant biomass, was increased by mycorrhizal colonization. Thus, no close relationship was found between enhanced P nutrition of inoculated plants and plant biomass. Mycorrhizal associations are often regarded as symbiotic systems in which host plants and mycorrhizal fungi rely upon and benefit from each other (Smith and Gianinazzi-Pearson 1988). However, the AM fungus may play the role of a plant parasite that can assist host plant nutrition and growth in some circumstances. When there is a ready supply of soil P for plant uptake, it may be energetically more economical for the plant to take up soil P directly than to take it up via the fungus and donate C substrates to the fungus, as reviewed by Jakobsen et al. (2002).

Under Cd contamination, both mycorrhizal colonization and P fertilization can decrease Cd partitioning to plant shoots. However, there may be differences in the underlying mechanisms besides the "dilution effect" as a result of improved plant P nutrition and growth. In mycorrhizal plants, binding of Cd by mycorrhizal structures may have lowered the partitioning of Cd to the shoots. At the Cd addition level of 100 mg kg^{-1} in the first experiment, the reduction in Cd translocation to the shoots with mycorrhizal association was less pronounced, perhaps because of toxicity from the relatively high level of added Cd.

Li and Christie (2001) suggested that depression of heavy metal translocation to plant shoots by mycorrhiza was associated with metal immobilization by the mycorrhizal fungi. The results of the second experiment are consistent with this hypothesis. The different effects of *G. mosseae* on Cd uptake by maize plants under the two Cd addition levels indicate that soil Cd contamination level had an important effect on the AM fungus. At the lower contamination level, the extraradical hyphae of the mycorrhizal fungus may have absorbed Cd from the hyphal compartment with some Cd transferred to the plants, resulting in increased Cd uptake by mycorrhizal plants compared with uninoculated controls. When the soil was more heavily contaminated, the high Cd concentration may have inhibited hyphal absorption, or may have inhibited fungal physiological activities, so that Cd was mainly adsorbed by or bound to the fungal mycelium.

Mycorrhizal colonization may have resulted in morphological or physiological changes in the roots (Atkinson et al. 1994), with subsequent changes in the mycorrhizosphere and effects on metal mobility and uptake by the plants. In the second experiment, soil pH in the hyphal compartment of inoculated treatments was generally higher than in the corresponding uninoculated treatments (data not shown). At the elevated soil pH levels the mobility and availability of Cd may have decreased (Xian and Shokohifard 1989), resulting in lower Cd uptake by the roots or extraradical hyphae.

The present study has provided further evidence for the inhibition of heavy metal translocation from roots to shoots in AM plants and effects of AM fungi on host plants under conditions of metal contamination. Non-mycorrhizal hyperaccumulator species that translocate metals efficiently to the shoots may be useful for phytoextraction of metals from the soil, but mycorrhizal plants that minimize metal translocation to the shoots may be useful for phytostabilization of contaminated sites (Leyval et al. 2002). Mycorrhizal crop species such as maize may also be grown in moderately contaminated areas without excessive metal transfer to the grain so that the soil can still be used for agricultural production without serious contamination of the food chain. However, further work is required to establish more accurately the role of mycorrhizal fungi in soil phytostabilization.

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