Mycorrhiza (2003) 13:151–157
DOI 10.1007/s00572-002-0211-8

ORIGINAL PAPER

R. Malcová · J. Rydlová · M. Vosátka

Metal-free cultivation of *Glomus* sp. BEG 140 isolated from Mn-contaminated soil reduces tolerance to Mn

Received: 10 June 2002 / Accepted: 15 October 2002 / Published online: 19 November 2002

Abstract The ability of arbuscular mycorrhizal (AM) fungi of different origin and cultivation history to tolerate excessive levels of manganese (Mn) was studied using hydroponic sand culture. Maize plants were colonised with two lineages of *Glomus* sp. BEG 140 from Mn-contaminated soil kept for 2 years in metal-free substrate or in the original soil. For comparison, the plants were also inoculated with *Glomus intraradices* BEG 75 from uncontaminated soil or were left uncolonised. Manganese stress was simulated by irrigation with nutrient solutions containing Mn at high concentrations (0.1, 0.5 and 1 mM); control plants were supplied with 3.8 μM Mn. Whereas the growth of maize plants was not suppressed by Mn at the concentrations examined, the development of AM fungi was negatively influenced by the higher Mn concentrations, with significant differences between isolates and cultivation lineages. The isolate *Glomus* sp. from Mn-contaminated soil showed higher tolerance to Mn than *G. intraradices* from uncontaminated soil. Colonisation by *G. intraradices* was reduced by almost 90% when irrigated with 1 mM Mn, whereas colonisation by the *Glomus* sp. lineage kept in contaminated soil still reached high levels (65% of the colonisation level of the control plants). The lineage of *Glomus* sp. cultured in inert metal-free substrate tolerated excessive Mn levels to a lesser extent than the lineage kept long-term in the original contaminated soil, but withstood Mn at higher concentrations than the *G. intraradices* from uncontaminated soil.

Keywords Arbuscular mycorrhizal fungi · Soil contamination · Heavy metal tolerance · Manganese toxicity

R. Malcová (✉) · J. Rydlová · M. Vosátka
Institute of Botany, Academy of Sciences of the Czech Republic, 252 43 Pruhonice, Czech Republic
e-mail: malcova@ibot.cas.cz
Tel.: +420-2-67750022
Fax: +420-2-67750022

Introduction

Manganese (Mn) availability in soils increases with decreasing pH and redox potential. Manganese toxicity can, therefore, occur in acid soils with large amounts of readily reducible Mn in combination with a high content of organic matter, high microbial activity and anaerobiosis (Marschner 1995). Concentrations of available Mn rarely reach toxic levels in agricultural fields (Davis 1996). Nevertheless, plant growth can be reduced in many acid soils without the development of toxicity symptoms (Arines et al. 1989). Alternatively, Mn toxicity can occur at waste disposal sites such as mine spoils or sedimentation ponds of metal smelters.

Excessive levels of Mn are toxic for AM fungi as has already been demonstrated by Hepper (1979), who observed a decline in the percentage of spore germination on agar containing Mn at high concentration. McGee (1987) reported significant inhibition of hyphal outgrowth from dried root pieces by Mn and found a negative correlation between the amount of Mn added to the soil and the percentage of root length colonised by AM fungi. In our earlier study (Malcová et al. 2002), we observed a significant decrease in the proliferation of hyphae from maize root segments colonised by *Glomus claroideum* BEG 23 at 0.05 mM Mn, with almost total inhibition at 0.5 mM Mn.

Species of AM fungi, and even various isolates of one species, can differ in their sensitivity to heavy metals (HM). Strains isolated from HM-polluted soils were found to tolerate higher concentrations of HM than reference strains from unpolluted soils (Gildon and Tinker 1983; Weissenhorn et al. 1994; Weissenhorn and Leyval 1995; del Val et al. 1999). Little is known, however, about the stability of HM tolerance during the cultivation of AM fungi in metal-free substrates (Meharg and Cairney 2000). Both persistent HM tolerance (Leyval et al. 1997) and loss of HM tolerance (Weissenhorn et al. 1994) have been reported.

Amelioration of Mn toxicity to plants by AM fungi has been observed and a protective role for AM fungi against

Mn toxicity in plants has been suggested (McGee 1987; Pacovsky 1986; Bethlenfalvay and Franson 1989). Lower Mn uptake by mycorrhizal than non-mycorrhizal plants has been reported in a number of studies (e.g. Pacovsky 1986; Arines et al. 1989). Manganese availability in the soil is dependent on microbial activity of Mn-oxidising and Mn-reducing rhizosphere microorganisms (Marschner 1995). Kothari et al. (1990) suggested that AM fungi depress Mn acquisition indirectly via changes in the microbial activity in the rhizosphere, since they affect the release of low-molecular-weight organic compounds from roots. Mycorrhizal plants were reported to inhibit Mn-reducing bacteria (Kothari et al. 1991; Posta et al. 1994) and promote Mn-oxidising bacteria in the rhizosphere (Arines et al. 1992), both resulting in decreased oxidation-reduction potential and reduced Mn availability in the mycorrhizosphere. However, some data indicate unchanged or even higher Mn uptake by mycorrhizal plants (Menge et al. 1978, 1982; Raju et al. 1987, 1990). Inconsistent results on the uptake of Mn by mycorrhizal plants may be related to highly variable soil conditions and the different AM isolates tested. It has been demonstrated that some AM fungi are more effective in reducing/enhancing Mn uptake by the plant than other AM species/isolates (Arines et al. 1989; Medeiros et al. 1994; Clark et al. 1999). Raju et al. (1990) described variability in Mn uptake by sorghum plants inoculated with different *Glomus* species. The plants inoculated with *Glomus macrocarpum* took up more Mn than uninoculated control plants, while the plants inoculated with *Glomus intraradices* showed either similar or even decreased Mn uptake compared with uninoculated plants. Liu et al. (2000) reported lower Mn contents in shoots of mycorrhizal than non-mycorrhizal plants at a higher dose of added micronutrients. At a lower dose of micronutrients or with no micronutrient addition, mycorrhizal and non-mycorrhizal plants had similar Mn contents.

In the present study, the toxicity of Mn for two AM isolates originating from Mn-contaminated or uncontaminated soil was investigated in two hydroponic experiments where Mn stress was simulated by irrigation with solutions containing Mn at different concentrations. Two lineages of the isolate from contaminated soil, cultivated long-term in the original or a metal-free substrate, were compared with respect to stability of Mn tolerance at increasing concentrations.

Materials and methods

AM fungi

The AM isolate *Glomus* sp. "cluster-forming" BEG 140, massively sporulating in roots similarly to *G. intraradices*, was isolated from a pyrite waste deposit Chvaletice (50°02'N, 15°26'E) in the Czech Republic in May 1996. The substrate is characterised by a fluctuating pH, high salinity and a high concentration of Mn (2,144 mg kg^{-1} ammonium acetate-EDTA extractable Mn, 255 mg kg^{-1} DTPA-$CaCl_2$-triethanolamine extractable Mn, 95 mg kg^{-1} $Ca(NO_3)_2$ extractable Mn). Since August 1998, the isolate *Glomus* sp. BEG 140 was cultivated separately as two lineages: in the original substrate (referred to as *Glomus* sp. OS) or in an inert metal-free substrate (clinoptilolite) (referred to as *Glomus* sp. IS). The lineages had been in cultivation for 2 years when experiment 1 was established. The generation time of *Glomus* sp. ranges from 3.5 to 6 months, depending on the season and substrate, both of which influence plant growth rate. The reference isolate, *G. intraradices* BEG75, originated from an unpolluted, agricultural area in Switzerland.

Experimental design

Experiment 1 involved 12 different treatments resulting from the combination of the following two factors: (1) Mn concentration (3.8 μM, 0.1 mM and 0.5 mM), and (2) inoculation (uninoculated, inoculated with *G. intraradices*, *Glomus* sp. OS or *Glomus* sp. IS). Seeds of maize (*Zea mays* L. cv. TATO-260) were surface-disinfected and pregerminated in Petri dishes. After 1 week, each seedling was planted in a 300-ml plastic tube filled with sterile quartz sand. Mycorrhizal treatments received 10 ml per plant of an inoculum suspension containing colonised root segments, extraradical mycelium (ERM) and spores. The non-mycorrhizal treatments received the same volume of autoclaved inoculum. In order to eliminate differences in microorganism populations introduced by AM inoculation, we attempted to restore soil microflora by irrigation of all treatments with a filtrate from the non-sterile mycorrhizal inocula. Soil microorganisms were isolated by passing a 1:10 suspension of soil inoculum through a filter paper to remove fungal spores and mycelia. Each treatment consisted of six tubes inserted into a plastic hydroponic container. For the first 2 weeks, the plants were supplied only with distilled water (1 l per container). Then, the plants were supplied twice a week with a P2N3 nutrient solution (Gryndler et al. 1992) containing corresponding Mn concentrations. The lowest Mn level (3.8 μM) corresponds to the basal Mn concentration in the nutrient solution P2N3. The two further Mn treatments received additional Mn (applied as $MnCl_2.4H_2O$). The pH of the nutrient solution was adjusted to 6.3 prior to application. The plants were grown in a greenhouse for 16 weeks (11 September 2000 to 3 January 2001). During the growth period, the positions of the containers with plants of different treatments were changed in the greenhouse once a week to minimise variance in cultivation conditions. After 16 weeks, all plants were harvested. Shoot and root dry weights were recorded after drying at 80°C. Subsequently, roots and shoots were ground, digested in HNO_3 and H_2O_2, and Mn concentrations were determined by atomic absorption spectrometry (Unicam 9200X; Allen et al. 1986). Root samples, for an evaluation of mycorrhizal colonisation, were stained with 0.05% trypan blue in lactoglycerol (Koske and Gemma 1989) and root colonisation was assessed using the grid-line intersect method (Giovanetti and Mosse 1980). The substrate from each tube was homogenised and small aliquots were taken to estimate the length of ERM using a modified membrane filtration technique (Jakobsen et al. 1992) and NADH diaphorase activity of ERM (Sylvia 1988; Hamel et al. 1990). A small sample of the substrate was placed in a household blender with 500 ml of distilled water and blended for 30 s. One millilitre of supernatant was pipetted onto a membrane filter (24-mm diameter, 0.40-μm pore size) and vacuum filtered. The mycelium retained on the surface of membrane filters was stained with 0.05% trypan blue in lactoglycerol. The total lengths of ERM were assessed using the grid-line intersect method under a microscope with an ocular grid at ×100 magnification and expressed in metres of hyphae in 1 g of air-dry substrate. The lengths of mycelium in uninoculated treatments were subtracted from the mycorrhizal treatment values. NADH diaphorase activity of the ERM was stained in the remaining ERM extracted from the substrate by wet sieving. NADH diaphorase staining solution (300 μl) was mixed with the mycelium sample in Eppendorf tubes and incubated at 28°C for 14 h in the dark. The proportion of ERM containing red precipitate (NADH diaphorase activity) was estimated under a microscope at a magnification of ×400.

In experiment 2, the inoculation treatments were the same (uninoculated, inoculated with *G. intraradices*, *Glomus* sp. OS or *Glomus* sp. IS) but a higher Mn concentration (1 mM) was used together with a control treatment (3.8 μM Mn) in order to test the results obtained from experiment 1. Each combination of treatments comprised 6 replicates. Cultivation conditions and the mode of irrigation were the same as in experiment 1. The plants were harvested after 16 weeks of growth (31 May to 20 September 2001) and plant growth and fungal development were assessed as described for experiment 1. As colonisation levels were very low in the majority of treatments supplied with Mn at higher concentrations, Mn uptake by plants was not evaluated.

Statistical treatment

The effects of inoculation and irrigation treatments were analysed using two-way analysis of variance (ANOVA). The effects of inoculation treatments within each Mn concentration were analysed by one-way ANOVA. Percent values of colonisation and NADH diaphorase activity were arcsine transformed prior to ANOVA. Comparisons between means were carried out using the Duncan's multiple range test at a significance level of $P<0.05$.

Results

Experiment 1

Both Mn irrigation and inoculation with AMF, as well as their interaction, had significant effects on the growth of maize plants (Table 1). Surprisingly, the plants treated with Mn-enriched nutrient solutions had overall higher dry weights than control plants. High Mn concentration, however, caused root browning, particularly with uninoculated plants and plants inoculated with *G. intraradices*. Of the plants treated with nutrient solution without additional Mn, uninoculated plants showed higher dry weights than plants inoculated with either of the AM isolates. At 0.1 mM Mn, the dry weights of maize shoots in different inoculation treatments followed the sequence: *Glomus* sp. OS inoculated and uninoculated plants > *Glomus* sp. IS inoculated plants > *G. intraradices* inoculated plants. At the highest level of Mn, the plants inoculated with either lineage of *Glomus* sp. had higher dry weights than either uninoculated plants or plants inoculated with *G. intraradices*.

At all three Mn levels, the concentrations of Mn in maize shoots were lower than in the roots (Table 2). A significant interaction between Mn irrigation and inoculation with AMF was found for Mn concentrations in the shoots and roots. Shoot Mn concentrations did not differ between inoculation treatments when no additional Mn was added. At higher Mn concentrations, the Mn concentrations in maize shoots were: plants inoculated with *G. intraradices* > plants inoculated with *Glomus* sp. IS > plants inoculated with *Glomus* sp. OS and uninoculated plants. Root Mn concentrations were lowest in plants inoculated with *G. intraradices* in the treatment without Mn amendment. At 0.1 mM Mn, root Mn concentrations did not differ between inoculation treatments. When treated with 0.5 mM Mn, root Mn concentrations were significantly higher in plants inoculated with *G. intraradices* than in the control. For plants inoculated with either lineage of *Glomus* sp., root Mn concentrations were not significantly different from control plants. The root to shoot ratio of Mn concentration increased with higher Mn levels (data not shown). In control treatments, the average root to shoot ratio of Mn concentration reached 2.2, while the values 2.9 and 5.9 were found when the plants were treated with 0.1 and 0.5 mM Mn, respectively. Considering differences between inoculation treatments, the root to shoot ratio of Mn concentration was highest for plants inoculated with *Glomus* sp. OS. When Mn concentration was recalculated and expressed as total Mn content, the effects of AM inoculation on Mn uptake by plants were different, since reduced metal

Table 1 The effects of manganese (Mn) and inoculation treatments on the growth of maize plants and on the development of the AM symbiosis in experiment 1. Means followed by the same letters are not significantly different within each Mn concentration according to Duncan's multiple range test at $P<0.05$ (*Glomus sp. IS* lineage kept in inert substrate, *Glomus sp. OS* lineage kept in original substrate, *ns* non-significant effect)

Mn (μM)	Inoculation	Shoot dry wt. (g)		Root dry wt. (g)		Root colonisation (%)		ERM length (m g^{-1} soil)		NADH diaphorase (%)	
3.8	Uninoculated	2.2	a	1.1	a	–		–		–	
3.8	*G. intraradices*	1.3	b	0.6	b	78	b	0.9	a	60	a
3.8	*Glomus* sp. IS	1.3	b	0.7	b	87	a	1.0	a	54	a
3.8	*Glomus* sp. OS	1.7	ab	0.7	b	88	a	0.9	a	57	a
100	Uninoculated	5.1	m	1.5	n	–		–		–	
100	*G. intraradices*	1.5	o	0.6	p	70	n	0.4	n	37	n
100	*Glomus* sp. IS	2.9	n	1.1	o	69	n	0.7	m	49	mn
100	*Glomus* sp. OS	6.2	m	2.0	m	86	m	0.9	m	52	m
500	Uninoculated	1.4	y	0.8	y	–		–		–	
500	*G. intraradices*	1.4	y	0.7	y	13	z	0.2	z	27	y
500	*Glomus* sp. IS	4.9	x	1.8	x	60	y	0.6	y	38	xy
500	*Glomus* sp. OS	5.3	x	1.6	x	74	x	0.8	x	49	x
Mn (A)		***		***		***		***		***	
Isolate (B)		***		***		***		***		**	
A × B		***		***		***		ns		*	

*Significant effect at $P<0.05$, **$P<0.01$, ***$P<0.001$

Table 2 The effect of Mn and inoculation treatments on Mn concentration (mg kg^{-1}) and content (mg per plant) in maize plants in Experiment 1. Symbols and abbreviations as in Table 1

Mn (μM)	Inoculation	Shoot concentration		Shoot content		Root concentration		Root content	
3.8	Uninoculated	55	a	114	a	103	ab	109	a
3.8	*G. intraradices*	50	a	64	b	63	b	35	b
3.8	*Glomus* sp. IS	66	a	76	b	164	a	108	a
3.8	*Glomus* sp. OS	61	a	106	a	176	a	125	a
100	Uninoculated	116	o	659	mn	319	m	460	n
100	*G. intraradices*	305	m	458	n	473	m	280	n
100	*Glomus* sp. IS	205	n	598	mn	425	m	482	n
100	*Glomus* sp. OS	136	o	840	m	598	m	1224	m
500	Uninoculated	145	yz	210	z	546	y	428	y
500	*G. intraradices*	356	x	496	y	1457	x	924	xy
500	*Glomus* sp. IS	193	xy	933	x	760	xy	1331	x
500	*Glomus* sp. OS	107	z	508	y	794	xy	1295	x
Mn (A)		***		***		***		***	
Isolate (B)		***		**		*		***	
A × B		***		***		**		**	

concentration was to some extent related to improved plant growth (Table 2).

Root colonisation was significantly reduced by high Mn concentrations, but this inhibitory effect differed between the AM isolates (Table 1). In the control treatment without Mn amendment, inoculation with either lineage of *Glomus* sp. resulted in a higher percentage root colonisation than *G. intraradices* inoculation. When irrigated with 0.1 mM Mn, the colonisation levels by *G. intraradices* and *Glomus* sp. IS were significantly lower than by *Glomus* sp. OS. The percentage root colonisation by *Glomus* sp. OS reached almost the same level as in the control treatment without Mn excess. Irrigation with 0.5 mM Mn decreased percentage root colonisation by *G. intraradices* by more than 80%, whilst colonisation by *Glomus* sp. OS and *Glomus* sp. IS was reduced by only 16% and 31%, respectively. The lengths of ERM decreased with increasing Mn concentration in the nutrient solution and differed also among AM isolates tested (Table 1). In the control treatment, ERM lengths were similar for all three AM isolates. At 0.1 mM Mn, ERM lengths were higher for both *Glomus* sp. lineages than for *G. intraradices*. At the highest Mn level, ERM length of *Glomus* sp. IS was significantly lower than that of *Glomus* sp. OS and higher than *G. intraradices*. NADH diaphorase activity in the ERM was negatively affected by higher Mn concentrations and Mn was more toxic for *G. intraradices* than for *Glomus* sp. (Table 1).

Experiment 2

Neither Mn concentration nor inoculation treatment influenced the growth of maize plants (Table 3). In contrast, the development of AM fungi was considerably reduced when plants were treated with 1 mM Mn. In the control treatment, the percentage root colonisation was AM species dependent. The plants inoculated with either lineage of *Glomus* sp. displayed relatively high levels of root colonisation, while the percentage root colonisation by *G. intraradices* was significantly lower. *Glomus* sp. also produced more ERM than *G. intraradices* in the control treatment. *G. intraradices* and the lineage of *Glomus* sp. grown for 2 years in an inert metal-free substrate were almost eliminated at 1 mM Mn and their colonisation levels were negligible. In contrast, *Glomus* sp. OS was able to withstand irrigation with 1 mM Mn and the root colonisation of plants inoculated with this lineage was only reduced from 81% to 53%. The negative effects of Mn concentration on ERM length also differed for each isolate. Irrigation with 1 mM Mn strongly reduced ERM length of *G. intraradices* and *Glomus* sp. IS, while ERM length of *Glomus* sp. OS was only slightly decreased. Irrigation with 1 mM Mn resulted in a substantial drop in the proportion of ERM showing NADH diaphorase activity. Almost no NADH diaphorase activity was detected for either *G. intraradices* or *Glomus* sp. IS. The NADH activity in ERM of *Glomus* sp. OS was also considerably decreased, but remained at 15% compared with 38% in the control treatment.

Discussion

Mycorrhizal colonisation of maize roots decreased with increasing Mn concentration in the nutrient solution. The inhibitory effect of higher Mn concentrations is in accordance with the results of previous studies dealing with the interaction of Mn and AM fungi (Hepper 1979; McGee 1987). In contrast, Schier and McQuattie (2000) observed an increase in root colonisation of sugar maple from approximately 25% at the control level of Mn (1.8 μM) to about 34% at 73 μM Mn; a decline in percentage colonisation was then observed at still higher Mn concentrations.

In the present experiment, root colonisation was already decreased at 0.1 mM Mn, whilst plant growth was even improved at this concentration. This stimulatory effect of higher Mn concentrations on maize growth confirms our previous results, where irrigation with 1 mM

Table 3 The effects of Mn and inoculation on the growth of maize plants and on the development of the AM symbiosis in experiment 2. Symbols and abbreviations as in Table 1

M (μM)	Inoculation	Shoot dry wt. (g)		Root dry wt. (g)		Root colonisation (%)		ERM length (m g^{-1} soil)		NADH diaphorase (%)	
3.8	Uninoculated	4.6	a	0.9	a	–		–		–	
3.8	*G intraradices*	4.4	a	1.1	a	16	c	1.1	b	42	a
3.8	*Glomus* sp. IS	4.2	a	0.9	a	67	b	2.1	a	39	a
3.8	*Glomus* sp. OS	3.6	a	0.9	a	81	a	1.9	a	38	a
1000	Uninoculated	4.2	x	1.1	x	–		–		–	
1000	*G. intraradices*	3.8	x	1.1	x	2	y	0.1	y	0	y
1000	*Glomus* sp. IS	4.0	x	1.0	x	2	y	0.2	y	0	y
1000	*Glomus* sp. OS	3.5	x	1.0	x	53	x	1.7	x	15	x
Mn (A)		ns		ns		***		***		***	
Isolate (B)		ns		ns		***		***		ns	
A × B		ns		ns		***		***		ns	

Mn resulted in a higher dry weight than in control maize plants (Malcová et al. 2002). Our results suggesting that plants have higher HM tolerance than AM fungi are in accordance with the findings of Gildon and Tinker (1983) and Weissenhorn and Leyval (1995). They observed a higher susceptibility to Zn and Cd with AM fungi than with onion and maize plants. Similarly, Liu et al. (2000) found that reduction in root colonisation by *G. intraradices* was higher than the reduction in maize plant growth at high levels of micronutrients.

The Mn concentration inhibiting root colonisation differed considerably between AM isolates with differing Mn exposure history. At increasing Mn, colonisation by the isolate from Mn-contaminated substrate did not decrease as dramatically as colonisation by the isolate from non-contaminated soil. These results correspond with several studies showing higher metal tolerance of AM isolates originating in contaminated sites than reference fungal strains (Gildon and Tinker 1983; Weissenhorn et al. 1993; Weissenhorn and Leyval 1995; del Val et al. 1999). Higher metal tolerance of isolates from polluted habitats has also been found for ectomycorrhizal and ericoid mycorrhizal fungi (Colpaert et al. 2000; Martino et al. 2000). On the other hand, some studies have provided evidence of strong intra- and interspecific variation in metal tolerance irrespective of isolate origin and metal-tolerant isolates have also been found among ectomycorrhizal and ericoid mycorrhizal fungi from non-polluted soils (Blaudez et al. 2000; Lacourt et al. 2000). Such extensive screening for metal tolerance in AM fungi has not yet been conducted, partly due to the obligately symbiotic nature of these fungi.

The decline in Mn tolerance of *Glomus* sp. BEG140 cultured in metal-free substrate, compared with the lineage grown constantly under metal stress in the original substrate, is in accordance with results of Weissenhorn et al. (1994). Using a simple germination test, they found out that spores from Cd- and Zn-tolerant cultures lost their higher metal tolerance after only one reproduction cycle (6 months) on metal-free substrate. In contrast, Leyval et al. (1997) reported that a metal-tolerant strain grown without metal stress retained its metal tolerance. However, these were only preliminary results and details of cultivation and subsequent testing of HM tolerance were not given. The evolution and stability of HM tolerance in AM fungi has not yet been elucidated (Leyval et al. 1997; Meharg and Cairney 2000). The latter authors supposed that genes encoding increased metal resistance are rapidly transferred through AM populations in the presence of selection pressures. When the selection pressure is removed, such genes are rapidly lost and return to low frequencies. Weissenhorn et al. (1994) described the development of Cd- and Zn tolerance in a fungal population treated for only 1 year with cadmium nitrate (40 mg Cd kg^{-1}). They hypothesised that such a rapid appearance of metal tolerance is based on phenotypic plasticity rather than on selection of tolerant genotypes, considering the relatively long generation time and large number of nuclei in the spores of AM fungi. Meharg and Cairney (2000) supposed that phenotypic plasticity is present in all populations, including those from uncontaminated environments. Our earlier results (Malcová et al. 2001) showing similar development of a native *G. claroideum* isolate from Mn-contaminated substrate and a reference strain (with no previous exposure to high Mn) in Mn-contaminated soil support this hypothesis.

Inconsistent effects of AM inoculation on the uptake of Mn by host plants correspond with reports on decreased, unchanged or increased Mn acquisition compared with non-mycorrhizal plants, depending on the AM species/isolates (Arines et al. 1989; Raju et al. 1990; Medeiros et al. 1994). In our experiment, unchanged or even increased shoot Mn concentrations (depending on the isolate and applied Mn concentration) were observed in inoculated compared with uninoculated plants, whilst total Mn contents of inoculated plants either decreased (at the control Mn level) or increased (at the highest Mn level). Since lower Mn concentrations observed in some inoculation treatments were related to higher shoot dry weights, this could partly be explained by dilution of the metal by improved plant growth. The same effect was described, for example, by Kucey and Janzen (1987), who reported lower Mn concentrations in inoculated plants.

However, increased dry matter of AM plants offset this effect, resulting in higher total Mn uptake by the larger inoculated plants. Similarly, Weissenhorn et al. (1995) reported that shoot and root Mn concentrations were lower in inoculated than in uninoculated maize plants. The total content did not differ, while inoculated plants displayed higher dry weights than those uninoculated.

We are aware that hydroponic sand culture receiving Mn applied in nutrient solution is a great simplification of the conditions in an original Mn-rich substrate. However, different edaphic stresses may be present in the substrate of origin of *Glomus* sp. and hydroponic cultivation enables us to separate the effects of a particular stress factor. The results obtained from artificial hydroponic cultivation need to be confirmed in the relevant naturally contaminated soils. The time aspect of tolerance loss also should be further studied in detail.

Acknowledgements This work was financially supported by the Ministry of Education, Youth and Sports of the Czech Republic within the EU program COST 8.38 and by the Institutional Research Concept AV0Z6005908. The authors are grateful to Claire Boddington for commenting on the English.

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