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ORIGINAL PAPER

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Response to cadmium of *Daucus carota* hairy roots dual cultures with *Glomus intraradices* or *Gigaspora margarita*

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Abstract Ri T-DNA-transformed carrot roots were cultivated in two experiments either non-inoculated or inoculated with the arbuscular mycorrhizal (AM) fungi *Glomus intraradices* or *Gigaspora margarita*. The influence of two concentrations of cadmium (Cd) in the medium (2 mg l^{-1}, 4 mg l^{-1}) on both root and mycelium growth was tested. Both parameters were estimated at 10-day intervals for 70 or 100 days for *G. intraradices* and *Gi. margarita*, respectively. In the first experiment, *G. intraradices* showed a rapid spread of extraradical mycelium (ERM) and reached average densities per treatment of about 90 cm cm^{-2} agar medium after 70 days. At the higher Cd level, the growth of ERM was delayed in comparison to the treatment without Cd addition. Root growth was inhibited by both Cd levels; the inhibition was, however, significantly lower in the treatments inoculated with *G. intraradices* compared to the non-inoculated control. In the second experiment, the ERM of *Gi. margarita* started to grow after a period of 50 days and reached average densities per treatment of only up to 27 cm cm^{-2} by the end of the cultivation. The growth of *Gi. margarita* mycelium was not inhibited by Cd. No differences in root growth were observed between the *Gi. margarita* inoculated and non-inoculated treatments. The inhibitory effect of Cd on root growth differed between the non-inoculated treatments in both experiments. The study has shown that the AM fungus *Glomus intraradices* can alleviate Cd-induced growth inhibition to carrot hairy roots. The potential and limits of the monoxenic system in studying the interaction between AM fungi and heavy metals are discussed.

Keywords Arbuscular mycorrhiza · Heavy metal · Monoxenic culture · Extraradical mycelium

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Introduction

Cadmium (Cd) is a heavy metal (HM) that naturally occurs in low concentrations in parent rocks and soils, and is not essential for plant nutrition. Cd concentrations in the environment can be substantially increased by human activities such as the use of phosphate fertilisers, application of sewage sludge, mining and smelting (Adriano 2001). In comparison with other HMs, the solubility of Cd in soils, as well as its toxicity to plants and animals, is high (Schachtschabel et al. 1992). Reduction of root growth is the first macroscopic symptom of Cd toxicity to plants (Seregin and Ivanov 2001).

Plant resistance to HM excess in soils can be achieved by mechanisms contributing either to the avoidance or to the tolerance of internal HM stress (Baker 1987). Among others, the symbiosis with arbuscular mycorrhizal fungi (AMF) has been suggested to confer benefit to plants growing in soils with toxic HM concentrations, though its contribution to plant resistance and the underlying mechanisms are as yet unclear (Leyval et al. 1997; Meharg and Carney 2000). Indication for an avoidance mechanism was given e.g. by Weissenhorn et al. (1995), who reported improved growth and decreased HM concentrations in tissues of AM-inoculated plants in comparison to non-inoculated plants in contaminated soil. However, AMF inoculation was also shown to improve plant growth without decreasing the HM concentrations in shoots (Dueck et al. 1986; Rivera-Becerril et al. 2002), or to have no effect at all on plant growth (Gildon and Tinker 1983). The contradictory results seem to be caused by the various factors that influence the response of the AM symbiosis to HMs in soil, such as plant species or even plant population (Griffioen and Ernst 1989; Hetrick et al. 1994; Díaz et al. 1996), AMF isolate (Hildebrandt et al. 1999; Malcová et al. 2003), HM concentration in soil (Heggo and Angle 1990; Díaz et al. 1996), irradiation (Weissenhorn et al. 1995) or root density (Joner and Leyval 2001).

The difficulties in studying AM symbiosis, resulting from the complex character of the soil environment and the obligate biotrophy of the fungal partner, can be partly

overcome by monoxenic cultivation of AMF on root-organ cultures (Fortin et al. 2002). In particular, the use of Ri T-DNA transformed roots and the medium designed by Bécard and Fortin (1988), offer a powerful tool, providing a simplified and controlled model system that can be observed non-destructively (Fortin et al. 2002). This enabled, among others, description of the development and architecture of the extraradical mycelium (ERM) (Chabot et al. 1992; Bago et al. 1998a,b), assessment of the effect of factors such as phosphate concentration in the medium on the development of the symbiosis (Olsson et al. 2002), and showed the ability of AMF to take up and translocate uranium (Rufyikiri et al. 2002).

At least 27 AM fungal species of five genera have been successfully cultivated on root-organ cultures (Fortin et al. 2002) and the life cycle of several species of the genus *Glomus* has been described (e.g. Chabot et al. 1992; Pawlowska et al. 1999; Karandashov et al. 2000; Declerck et al. 2001). The in vitro system can therefore be used for comparing species of different AMF genera that have been shown to differ in pot cultures with plants in e.g. their colonisation strategies (Hart and Reader 2002; Klironomos and Hart 2002) or in their response to phosphate (Boddington and Dodd 1999).

The aim of the present study was to monitor the effect of two Cd concentrations on the development of in vitro symbiosis between Ri T-DNA-transformed carrot roots and two AMF species from the genera *Glomus* and *Gigaspora*. We tested whether AM symbiosis affects the growth and Cd uptake of the root-organ culture in comparison with non-inoculated roots.

Materials and methods

Two experiments of the same design were established for the two fungi studied, *Glomus intraradices* Schenck and Smith, isolate BEG75, and *Gigaspora margarita* Becker and Hall, isolate BEG34. The Ri T-DNA-transformed roots of carrot (*Daucus carota* L.), clone DC1, were kindly provided by B. Bago.

Experiment I: *Glomus intraradices* BEG75

Simple round plastic Petri plates (diameter 9.5 cm) were filled with 40 ml minimal medium (M medium, Bécard and Fortin 1988) pH 5.5 with 0.4% (w/v) Phytagel (Sigma, St. Louis, Mo.) as gelling agent. The medium was amended with 0, 2 or 4 mg Cd l^{-1} as $Cd(NO_3)_2$, where the higher Cd level was chosen according to results of preliminary tests on Cd toxicity to the root material. Five Petri plates of each Cd treatment were inoculated with a core of M medium containing colonised roots, ERM and spores from a growing monoxenic culture of *G. intraradices* on Ri T-DNA-transformed carrot roots. A core of axenic carrot roots growing on the same medium was applied to another five Petri plates for each Cd treatment. A piece (~20 mm) of actively growing root was added to each plate. The plates were inverted and incubated at 25°C in the dark.

Root length was estimated by counting intersections (Newman 1966) on a 1×1 cm grid covering the whole plate under a stereomicroscope every 10 days beginning on day 20. The length of ERM was recorded in the same intervals under a microscope (100× magnification), on 50 (lower mycelium density at the beginning of the cultivation) or on 15 (higher mycelium density) grids (10×0.01 cm) covering the plate at regular distances (modified method for ERM length quantification described by Brundrett et al. 1994). After 70 days, roots were extracted by shaking the whole contents of the plate for 1 h in 10 mM Na-citrate buffer pH 6.0 (Doner and Bécard 1991), and total root dry weight was determined for every plate after drying at 80°C. Root sub-samples of about 0.8 g fresh weight were stained with 0.05% Trypan Blue in lactoglycerol (Koske and Gemma 1989) and root colonisation was assessed by the grid-line intersect method (Giovanetti and Mosse 1980). The rest of the roots was ground, decomposed by a dry ashing procedure, and the ash was dissolved in 1.5% nitric acid. The Cd concentrations in the roots were determined by atomic absorption spectrometry (Varian SpectrAA-300; Varian, Harbor City, Calif.).

Experiment II: *Gigaspora margarita* BEG34

Plates were prepared in the same way as for experiment I, except that all plates were provided with a core of axenic carrot roots, and each plate of the mycorrhizal treatments was inoculated with five surface-sterilised (Jansa et al. 2002), not pre-germinated spores. The spores had been extracted by wet sieving from a *Gigaspora margarita*-maize pot culture. The incubation conditions, as well as the observation of root and mycelium growth, were the same as in experiment I except that mycelium growth was always assessed on 50 grids. Due to the slower development of the fungus, the experiment was harvested after 100 days, and the same parameters as in experiment I were evaluated.

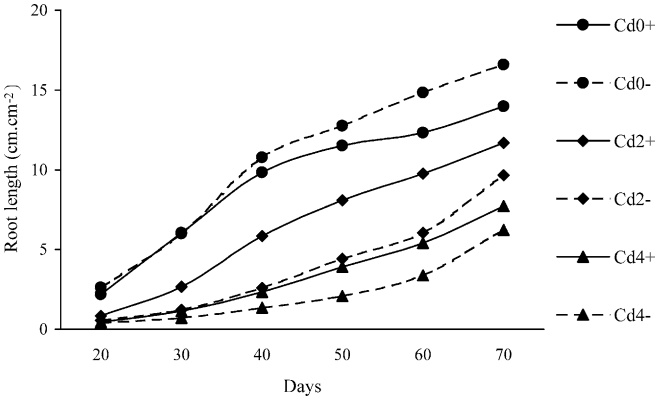

Fig. 1 The development of carrot hairy root cultures as cultivated on M medium with different Cd levels (0, 2 or 4 mg l^{-1}). − non-inoculated, + inoculated with *Glomus intraradices*

Table 1 Experiment I: the effects with *F*-values of cadmium (Cd) (*A*; *column 2*) and inoculation with*Glomus intraradices*(*B*) on growth of carrot hairy roots on M medium, and the effects of Cd (*A*; *column 5*) on extraradical mycelium (ERM) growth of *G. intraradices*. Two-way and one-way ANOVA were applied to root length and ERM length data from each measurement. The effects of the factors for the whole observation period, their interactions with the factor time and the effects of time are given according to repeated measures ANOVA

Day	Root length			ERM length
	A	B	A×B	A
20	39.99***	0.16 ns	0.71 ns	
30	64.81***	7.41*	2.03 ns	3.00 ns
40	73.12***	11.60**	5.18*	5.51*
50	48.01**	10.56**	4.10*	4.40*
60	35.46***	6.49*	4.25*	1.22 ns
70	37.72***	0.15 ns	3.51*	0.95 ns
Whole period	59.99***	7.09*	3.62*	2.88 ns
Interaction with time	11.73***	6.14**	2.30*	2.22 ns
Effect of time	Root length 637.77***			ERM length 47.10***

***P<0.001; **P<0.01; *P<0.05; ns non-significant

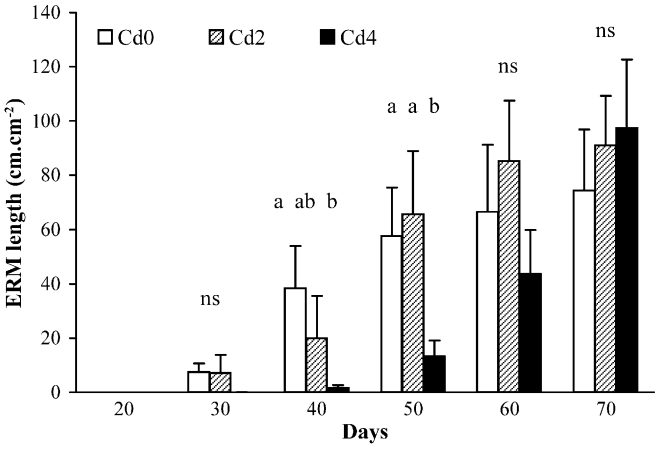

Fig. 2 The length of the extraradical mycelium (ERM) of *G. intraradices* at 10-day intervals as cultivated with carrot hairy roots on M medium with different Cd levels (0, 2 or 4 mg l^{-1}). *Bars* are means (+SE). Means within one observation time marked with different letters are significantly different according to Duncan's multiple range test at P<0.05; *ns* non-significant effect of Cd at the level P<0.05

Statistical treatment

Data were logarithmically [ln(x+1)] transformed where necessary in order to obtain normal distribution, and analysed using one-way or two-way analysis of variance (ANOVA). Comparisons between means were carried out using the Duncan's multiple range test at the significance level of P<0.05. The effect of time on root length was evaluated by repeated measures ANOVA.

Results

Experiment I: *Glomus intraradices*

Cd in the medium significantly decreased root length throughout the whole observation period (Fig. 1, Table 1) as well as the root dry weight at harvest (Table 2). However, root length and root dry weight were also significantly influenced by inoculation with *G. intraradices* and by the interaction of the factors Cd in the medium and inoculation (Tables 1, 2). The interaction was significant for root length values from day 40 on, as well as for the root dry weight, and consisted in higher values for inoculated than for non-inoculated roots in the treatments with Cd amendment, but not in the control treatments without Cd (Fig. 1). The effect of Cd and inoculation on root length changed with time as both factors significantly interacted with the time factor (Table 1). The effect of Cd on root length declined in both the inoculated and non-inoculated treatments: the average root length of the mycorrhizal treatment at 4 mg l^{-1} Cd, for example, was decreased by 82% at day 30, whereas by only 45% at day 70 in comparison to the treatment without Cd amendment.

The growth of ERM of *G. intraradices* started between day 20 and 30, and on day 70, average ERM length per treatment ranged between 74 and 97 cm hyphae per square centimetre (Fig. 2). The variability in ERM growth was high within the Cd treatments, but a delay in ERM growth

Table 2 The effects with *F*-values of Cd and inoculation with *G. intraradices* on root dry weight, Cd concentration in roots and Cd content in roots per plate of carrot hairy root cultures on M medium in experiment I. Values are means ±SE. Means in columns followed by the same letters are not significantly different according to Duncan's multiple range test at P<0.05

Cd (mg l^{-1})	Inoculation	Root dry weight (mg)	Cd concentration (μg g^{-1})	Cd content (μg)
0	+	74.8±3.06 a	2.9±0.65 d	0.22±0.05 c
0	−	80.6±4.43 a	0.4±0.08 d	0.03±0.01 c
2	+	62.6±2.62 b	297.2±16.20 c	18.56±1.06 b
2	−	45.4±3.17 c	442.8±91.64 c	20.04±4.27 b
4	+	44.8±2.86 c	629.6±53.61 b	27.65±1.04 a
4	−	26.4±5.46 d	1,201.8±90.33 a	31.51±7.13 ab
F-value and significance of 1-way ANOVA		16.52***	62.16***	15.17***
F-values and significant effects of 2-way ANOVA				
Cd (A)		63.51***	128.85***	37.58***
Inoculation (B)		10.60**	25.98***	0.37 ns
A×B		6.67**	13.56***	0.17 ns

***P<0.001, **P<0.01, *P<0.05; ns non-significant

Table 3 Percentage of root length and total root length colonised by *G. intraradices* and *Gigaspora margarita* in experiments I and II, respectively. Values are means ±SE. Means in columns followed by the same letters are not significantly different according to Duncan's multiple range test

Cd (mg l^{-1})	*G. intraradices* (Experiment I)		*Gi. margarita* (Experiment II)	
	Colonisation (%)	Colonised root length (cm cm^{-1})	Colonisation (%)	Colonised root length (cm cm^{-1})
0	5.7 (±2.1) b	0.8 (±0.3) a	5.4 (±1.6) a	0.7 (±0.2) a
2	13.2 (±1.4) ab	1.5 (±0.1) a	5.9 (±2.3) a	0.9 (±0.4) a
4	22.3 (±6.7) a	1.9 (±0.6) a	8.1 (±1.9) a	1.1 (±0.3) a
F-value and significance	4.07*	1.91 ns	0.55 ns	0.53 ns

***$P<0.001$, **$P<0.01$, *$P<0.05$; ns non-significant

was apparent in the treatment with 4 mg l^{-1} Cd, as ERM length was significantly lower than in the control treatment without Cd on days 40 and 50 (Table 1, Fig. 2). The percentage of root colonisation by *G. intraradices* was significantly lower in the control treatment without Cd than in the treatment with 4 mg l^{-1} Cd, but the length of colonised roots did not differ between the Cd treatments (Table 3).

Cd concentrations in roots at harvest were significantly increased by Cd addition to the medium and affected by both inoculation and the interaction between the factors Cd and inoculation (Table 2): at 4 mg l^{-1} Cd, Cd root concentration was significantly lower in the inoculated than in the non-inoculated treatment. The total Cd content in roots per plate, however, did not differ between the mycorrhizal and non-mycorrhizal treatments (Table 2).

Experiment II: *Gigaspora margarita*

The influence of Cd addition and inoculation with *Gi. margarita* on root growth was substantially different compared to experiment I with *G. intraradices* (Fig. 3). Cd in the medium decreased root length until day 50 of the cultivation only, and from day 60 on, there was no significant difference in root length between the Cd treatments (Table 4). The decreasing effect of Cd on root length is also reflected in the interaction of this factor with time (Table 4). No

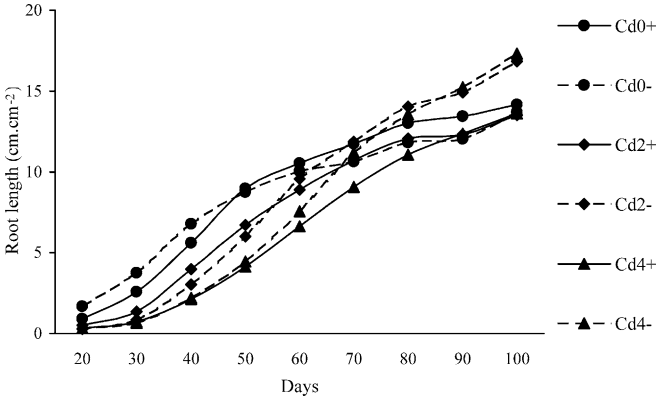

Fig. 3 The development of carrot hairy root cultures as cultivated on M medium with different Cd levels (0, 2 or 4 mg l^{-1}). − non-inoculated, + inoculated with *Gigaspora margarita*

significant effect of inoculation on root length was found at any observation time. The root dry weight at harvest was significantly influenced by the interaction of Cd level and inoculation (Table 5).

The ERM development of *Gi. margarita* was characterised by a rapid germination and growth of germinating hyphae already in the first week, followed by a long lag-phase before ERM growth started again between days 50 and 60 of the cultivation (Fig. 4). The average ERM density per treatment reached values of between 20 and 27 cm cm^{-2} at day 100, much lower than those obtained for *G. intraradices* in experiment I at day 70. The variability in ERM length within Cd treatments was high and no significant difference in ERM length was recorded between the Cd treatments at any observation time (Table 4). Neither the percentage of root colonisation by *Gi. margarita* nor the total length of colonised roots differed significantly between the Cd treatments (Table 3).

Table 4 Experiment II: the effects and *F*-values of Cd (*A*; *column 2*) and inoculation with *Gi. margarita* (*B*) on growth of carrot hairy roots on M medium, and effects of Cd (*A*; *column 5*) on ERM growth of *Gi. margarita*. Two-way and one-way ANOVA were applied to root length and ERM length data from each measurement. The effects of the factors for the whole observation period, their interactions with the factor time and the effects of time are given according to repeated measures ANOVA

Day	Root length			ERM length
	A	B	A×B	A
20	17.41***	0.28 ns	3.31 ns	2.11 ns
30	22.60***	0.10 ns	1.90 ns	0.55 ns
40	13.99***	0.29 ns	0.63 ns	0.69 ns
50	8.31**	0.12 ns	0.07 ns	0.74 ns
60,70,80, 90,100	<3.05 ns	<3.14 ns	<1.31 ns	<0.76 ns
Whole period	5.02*	0.15 ns	0.10 ns	0.24 ns
Interaction with time	15.16***	1.45 ns	2.87*	0.75 ns
Effect of time	Root length 787.67***			ERM length 31.96***

***$P<0.001$, **$P<0.01$, *$P<0.05$; ns non-significant

Table 5 The effects with *F*-values of Cd and inoculation with *Gi. margarita* on root dry weight, Cd concentration in roots and Cd content in roots per plate of carrot hairy root cultures on M medium in experiment II. Values are means ±SE. Means in columns followed by the same letters are not significantly different according to Duncan's multiple range test at $P<0.05$

Cd (mg l^{-1})	Inoculation	Root dry weight (mg)	Cd concentration (μg g^{-1})	Cd content (μg)
0	+	83±4.03 ab	1.4±0.58 c	0.12±0.05 c
0	−	70±4.45 c	0.5±0.22 c	0.04±0.02 c
2	+	72±3.54 bc	299.0±17.35 b	21.54±1.28 b
2	−	82±4.79 ab	381.0±47.32 b	29.70±1.26 b
4	+	77±2.54 abc	529.0±78.81 a	39.00±6.63 a
4	−	86±3.09 a	518.0±15.53 a	42.99±1.69 a
F-value and significance of 1-way ANOVA		2.86*	143.31***	41.98***
F-values and significant effects of 2-way ANOVA				
Cd (A)		1.17 ns	93.79***	102.47***
Inoculation (B)		0.43 ns	0.55 ns	2.91 ns
A×B		5.78**	0.87 ns	1.02 ns

***$P<0.001$, **$P<0.01$, *$P<0.05$; ns non-significant

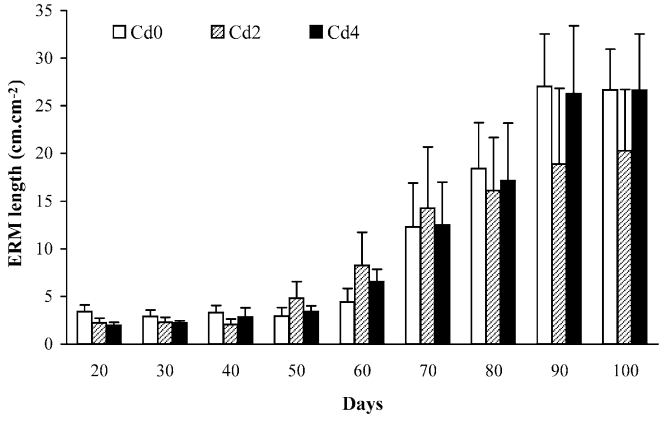

Fig. 4 The length of the ERM of *Gi. margarita* at 10-day intervals as cultivated with carrot hairy roots on M medium with different Cd levels (0, 2 or 4 mg l^{-1}). *Bars* Means +SE. No significant effects of Cd were found for any observation in time at the level $P<0.05$

The Cd concentrations in roots and the Cd contents in roots per plate were influenced by Cd level in the medium only (Table 5). Inoculation had no effect on the Cd concentration and content in the roots.

Discussion

In experiment I, the DC1 clone of carrot hairy roots was clearly more sensitive to Cd in the medium than *G. intraradices*, as Cd more pronouncedly inhibited root growth than ERM growth of *G. intraradices*, and root colonisation by the fungus was not at all inhibited by Cd. Moreover, the observed delay in ERM development of *G. intraradices* at the higher level of Cd amendment cannot be unambiguously attributed to the direct toxic effect of Cd. The development of a monoxenic AMF culture depends on the growth and physiological state of the host roots (Bago et al. 1998a; Fortin et al. 2002). It is therefore possible that the effect of Cd on the dynamics of ERM development was indirect, mediated through the effect on root growth.

Rivera-Becerril et al. (2002) found that a *G. intraradices* isolate, which had not been previously exposed to Cd, was more tolerant to Cd than three tested genotypes of pea. They attributed their results to a generally high HM tolerance of this AMF species, indicated also by the findings of Jacquot et al. (2000), who compared the HM tolerance of *G. intraradices* and two other AMF species. In our experiment II, however, neither germination nor proliferation of germinating hyphae of *Gi. margarita* were affected by Cd in the medium at levels that inhibited root growth. Weissenhorn et al. (1993) demonstrated a decrease in spore germination and growth of germinating hyphae of *Glomus mosseae* at Cd concentrations in solution between 0.5 and 5 mg l^{-1}. Bartolome-Esteban and Schenck (1994), on the other hand, showed that the spore germination of *Gigaspora* species is more tolerant to aluminium ions than that of *Glomus* species, though representatives of the two genera have not been compared in the symbiotic phase. Nevertheless, our results indicate that carrot hairy roots may be generally more sensitive to Cd than AMF under the given culture conditions.

The effect of Cd on root and ERM growth seems to have consisted mainly in the prolongation of the lag-phase. On the other hand, the decreasing effect of Cd on root length in the course of the cultivation period may also indicate decreasing amounts of free Cd in the medium due to Cd sorption in the system. As reported earlier, M medium acidifies in later stages of root culture development (Bago et al. 1996; Karandashov et al. 2000; Villegas and Fortin 2001). No increased sorption to medium components, or precipitation with the latter, was therefore probable, due to the increasing solubility of Cd with decreasing pH (Adriano 2001). Cd sorption and uptake by roots and ERM, or complexation by root exudates, are therefore the factors that may have decreased the toxic effect of Cd in the cultivation system.

The ability of root exudates to ameliorate the inhibitory effects of lead and manganese to hyphal growth of *G. intraradices* has been demonstrated by Malcová and Gryndler (2003) in a simplified in vitro system. It is therefore probable that root exudates accumulating in the cultivation system decreased the amount of free Cd ions in the cultivation medium. In the treatments with 4 mg l^{-1} Cd, roots contained almost 20% of the initially added Cd at the end of the cultivation period. At 2 mg l^{-1} Cd, this rate was about 25%.

We do not know how much of the added Cd was bioavailable at the beginning of the cultivation, but the proportion must have been higher in the treatments with higher Cd concentrations in the medium. The higher rate of root-extracted Cd in the treatments with lower Cd concentrations in the medium indicates therefore that, although roots bound a considerable proportion of the added Cd at the end of the cultivation, they did not immobilise all bioavailable Cd, at least in the treatments with 4 mg l^{-1} Cd.

The alleviation of the inhibitory effect of Cd on root growth by inoculation with *G. intraradices* indicates that fungal structures may have played a role in decreasing the amount of free Cd in the cultivation system. The ERM of AMF has been shown to have a high sorption capacity for HMs, and HM sorption on ERM was therefore suggested as a mechanism by which AM symbiosis could decrease the HM uptake of plants and consequently alleviate HM stress (Joner et al. 2000; Gonzalez-Chavez et al. 2002). In our study, however, inoculation with *G. intraradices* did not decrease the Cd content in roots per plate (i.e. the total Cd uptake from the medium by the root culture), although the Cd concentration was lower in mycorrhizal roots with higher biomass than in non-mycorrhizal roots with lower biomass. This fact does not indicate a significant decrease in Cd bioavailability in the plates inoculated with *G. intraradices*. Consequently, Cd immobilisation in the medium by the fungus was probably not the main mechanism for the amelioration of the toxic effect of Cd to the roots.

Other mechanisms must therefore have contributed to the alleviation of Cd toxicity to roots. Sequestration of HMs in fungal intraradical structures has been suggested by Turnau et al. (1993). This process would not alter the Cd concentrations in roots of mycorrhizal plants, but would decrease their Cd assimilation as compared to non-mycorrhizal plants. Repetto et al. (2003) have shown that AM symbiosis can simultaneously alleviate Cd toxicity to pea and modulate the expression of proteins involved in Cd-response in pea roots—an effect of AM that has not yet been studied in more detail. Finally, Meharg and Cairney (2000) attribute growth improvements of mycorrhizal vs. non-mycorrhizal plants to nutritional effects similar to those in uncontaminated environments. In plants, Cd interferes with the uptake, transport and use of water and nutrients such as Ca, Mg, P and K (Das et al. 1997). AM symbiosis may consequently protect plants against HM toxicity by improving their nutrient uptake if root functionality is decreased by Cd damage, while the symbiosis has no effect on plant growth in normal conditions of fully functional roots.

The development of the cultures followed a different pattern in experiment II with *Gi. margarita* than in experiment I with *G. intraradices* in several features. The exponential growth of the ERM of *Gi. margarita* began later, and ERM had much lower densities after 100 days of cultivation despite having reached the stationary phase of logarithmic growth at that time. The fast growth of hyphae from germinating spores indicates that the delay was not caused by inappropriate inoculum, but rather by slow root colonisation and/or late re-growth of secondary mycelium from infected roots. This corresponds to the slower development of *Gigaspora* species in comparison to *Glomus* species found by Boddington and Dodd (1998) and Hart and Reader (2002) in pot cultivation, and to the observations of Tiwari and Adholeya (2002), who co-cultivated *G. intraradices* and *Gi. margarita* in-vitro. On the other hand, higher ERM densities have been reported from later stages of pot cultivation for *Gigaspora* species than for *Glomus* species (Boddington and Dodd 1998; Hart and Reader 2002). This indicates that differences in life strategies between the species of both genera observed in pot cultivation are not always reflected in the monoxenic system.

The major difference between experiments I and II, however, consisted in the development of roots. The inhibitory effect of Cd on root growth was much lower in experiment II, in both the inoculated and non-inoculated treatments. Cd concentrations in the medium, medium composition, and culture conditions were the same in both experiments. The amount of Cd extracted from the medium by roots at the end of the cultivation (i.e. the Cd content of roots) was even higher in experiment II than in experiment I, which argues against lower Cd bioavailability in the systems of experiment II due to some unknown factor. The large difference in Cd root concentrations between the 4 mg l^{-1} Cd treatments of experiment I and experiment II corresponds to the higher root biomass in experiment II vs. experiment I. The most probable explanation is, therefore, that root material was responsible for the observed differences. This assumption is also supported by the fact that the growth of non-inoculated roots differed between the experiments in all Cd treatments: in experiment I, roots colonised the plates faster when no Cd was added to the medium, but slower on Cd-amended plates. Roots of the same clone can differ in their behaviour even if grown in the same conditions (Fortin et al. 2002) and the comparison between experiments I and II suggests that the physiological state of the root material is of great importance for the reproducibility of results obtained in monoxenic cultivation.

The effect of both fungi on root growth under conditions of Cd stress cannot be compared due to the differences in root growth in the two experiments. The distinct ERM growth patterns of both fungi, however, indicate that differences must be expected if ERM plays a role in the amelioration of Cd toxicity to roots.

In conclusion, this study has shown some of the potential and the limits of the monoxenic system in the study of AM and HMs. The limits of the system consisted especially in the low tolerance of carrot hairy roots to Cd and the possible decrease in the amount of free Cd ions in the medium during the cultivation period. More sophisticated experimental units, such as compartmented plates (St-Arnaud et al. 1995), are therefore required to study the direct effect of HMs on the fungal symbiont. Moreover, the importance of the response of the roots for the reproducibility of results shows that careful standardisation of root material is necessary for experimental series in the monoxenic system. Nevertheless, the simple monoxenic system allowed comparison of ERM development of *G. intraradices* and *Gi. margarita*, and can be used for

more detailed studies on differences between the two genera. Furthermore, it has been shown that the AM symbiosis can alleviate Cd stress to plant roots in the case of *G. intraradices* and provides a tool for studying the mechanisms of this stress alleviation.

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