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Arbuscular mycorrhizal fungi can decrease the uptake of uranium by subterranean clover grown at high levels of uranium in soil

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"Capsule": *Plant mycorrhization may decrease U concentration in shoots of plants grown at high level of U in soil.*

Abstract

Subterranean clover inoculated or not with the arbuscular mycorrhizal (AM) fungus *Glomus intraradices* was grown on soil containing six levels of ^{238}U in the range 0–87 mg kg^{-1}. Increasing U concentration in soil enhanced the U concentration in roots and shoots of both mycorrhizal and nonmycorrhizal plants but had no significant effects on plant dry matter production or root AM colonization. Mycorrhizas increased the shoot dry matter and P concentration in roots and shoots, while in most cases, it decreased the Ca, Mg and K concentrations in plants. The AM fungus influenced U concentration in plants only in the treatment receiving 87 mg U kg^{-1} soil. In this case, U concentration in shoots of nonmycorrhizal plants was 1.7 times that of shoots of mycorrhizal plants. These results suggested that mycorrhizal fungi can limit U accumulation by plants exposed to high levels of U in soil.

Keywords: ^{238}U; Mycorrhizal fungi; Uptake; Radionuclides; Plant

1. Introduction

Uranium (U) is present in most continental earth's crust soils as a natural trace element with an average abundance of 2.6 mg kg^{-1} soil (Taylor, 1964), but in local areas, it can reach concentrations as high as tens or hundreds mg kg^{-1} soil, mainly in U ore deposits (Plant et al., 1999). Mining and milling of U ores are the most important sources of U contamination of the environment because they generate large quantities of waste materials stored up as heaps of mining rock debris or dumps of mill wastes after U processing. Improper storage of U wastes can result in U dispersion on soil surface by runoff, in the air by wind and in groundwater by leaching. For instance, U concentration up to 275 mg kg^{-1} was observed in U tailings in Germany, and while the U concentration was 0.6 mg l^{-1} for groundwater flowing into the tailings, its concentration in groundwater within the tailings and groundwater leaving the tailings was 707 and 260 mg l^{-1}, respectively (Junghans and Helling, 1998).

Like other heavy metals U is chemically toxic, and it can also pose radiological hazards. Therefore, there is health risks for both humans and animals exposed to U-contaminated environments, and the ingestion of U by drinking U-contaminated water or via the food chain constitutes an important pathway of U exposure for

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humans. To limit U dispersion in the environment, different remedial actions including physical, chemical and biological methods have been proposed (Abdelouas et al., 1999; Suzuki and Banfield, 1999). Among all these techniques, phytoremediation consisting in the use of plants and associated microorganisms was considered as the most suitable method for long-term rehabilitation of U contaminated sites, because it is not destructive for the environment (Huang et al., 1998; Shahandeh et al., 2001). In this respect, it is essential to know the contribution of the different partners involved in the association, i.e. plants and microorganisms, in the U uptake by plants, and to understand the mechanisms involved, whatever the phytoremediation (phytoextraction or phytostabilization) option advocated.

Plant roots are associated with microorganisms that can have either direct or indirect effects on the mobility, availability and uptake of elements by plants. Among the soil microflora, arbuscular mycorrhizal (AM) fungi are involved in the most widely distributed root symbioses (Smith and Read, 1997), forming association with most terrestrial flowering plants (Harrison, 1997). In this symbiosis, the fungus receives from the host plant carbohydrates necessary for its growth. In return, improved mineral nutrition (Jakobsen et al., 2002; Ryan and Angus, 2003) and increased tolerance/resistance against toxic elements (Cumming and Ning, 2003; Rufyikiri et al., 2000), root pathogens (Declerck et al., 2002) and water deficit stress (Borkowska, 2002; Porcel et al., 2003) are some of benefits for the host plants. AM fungi could also influence radionuclide acquisition by plants, as it was reported for in vivo studies involving ^{137}Cs (Entry et al., 1999; Berreck and Haselwandter, 2001), ^{134}Cs (Strandberg and Johansson, 1998) and ^{90}Sr (Entry et al., 1999), and more recently for an in vitro study involving ^{137}Cs (Declerck et al., 2003). Another in vitro study using root-organ culture demonstrated that the extraradical fungal mycelium can take up and translocate ^{233}U towards plant roots although in small quantities (Rufyikiri et al., 2002, 2003, in press), and both processes were highly influenced by the pH of the growth medium. The in vitro experimental approach had as major limitation in not considering the role of shoots as sink and the interferences with soil components which may have strong effects on U bioavailability. The AM fungal effect on the uptake of toxic elements by plants can also vary in function of levels of these elements in the growth medium as it was reported for Al (Rufyikiri et al., 2000) and Pb (Malcova et al., 2003). At high concentrations of heavy metals and of any nonessential metals, AM fungi often improve plant tolerance/resistance against toxic effects by increasing metal retention in the roots thereby reducing metal concentration in shoots (Leyval and Joner, 2001). Such effects have not yet been studied for uranium.

The present study aimed to determine the role of the AM fungus *Glomus intraradices* in the U acquisition by plants in vivo. The AM fungal effects on U uptake by plants were determined at a wide range of levels of U contamination of the soil.

2. Materials and methods

2.1. Biological material

Seeds of subterranean clover (*Trifolium subterraneum* L., cv. Mount Barker) were surface-sterilized by immersion in 2 M H_2O_2 for 10 min, rinsed in deionized water and germinated on wet tissue for 48 h before sowing. The source of inoculum was an air-dried and sieved (<2 mm) soil collected from 2 month-old pure culture pot of subterranean clover inoculated with *Glomus intraradices* Schenk and Smith (isolate 28A, BEG 87).

2.2. Phase of mycorrhization

For mycorrhizal treatment (+AM treatment), 30 g of sterilized non U-contaminated soil described below were mixed with 40 g of inoculum soil, watered to the field capacity and placed in open 100-ml pots. The inoculum soil was sterilized by gamma-irradiation to have a control nonmycorrhizal treatment (−AM treatment). Four pre-germinated seeds were sown into each pot and thinned out to two plants after emergence. The plants were maintained in greenhouse with 16/8 h light/dark cycles, a temperature varying between a minimum of 15 °C during the night and a maximum of 29 °C on sunny days, a relative humidity varying between 15–74%. The light intensity was in the range 438–1880 μmol m^{-2} s^{-1} photon flux density and the minimum was supplied by Philips SON-T 400 daylight lamps (380–779 nm) with an automatic regulation, while the daily maxima were reached on sunny days. Plants were watered daily by weight. After 4 weeks, root samples were taken from pots and analysed for AM fungal colonization as described below.

2.3. Soil and U contamination

The soil was collected from grassland in Kalmthout, Belgium (51° 22′ 59.0″ N 04° 29′ 33.0″ E). Its main characteristics determined according to the methods described below are given in Table 1. After drying at room temperature and sieving (<2 mm), the soil was sterilized by gamma-ray irradiation (25 kGy) in order to eliminate the indigenous mycorrhizal fungi.

Six levels of U were added to the soil: 0, 5, 8, 20, 42 and 87 mg ^{238}U kg^{-1} dry soil. The source of U was a salt of uranyl acetate [$UO_2(CH_3COO)_2{\cdot}2H_2O$] supplied by

Table 1
Main chemical characteristics of the soil used

Variables	Values
pH-H_2O (1:5 soil/water ratio)	5.18 ± 0.06
pH-KCl (1:5 soil/1M KCl ratio)	4.38 ± 0.02
Total P (mg kg^{-1} soil)	940 ± 10
Olsen-P (mg kg^{-1} soil)	79.8 ± 0.9
Water-soluble P (mg kg^{-1} soil)	0.08 ± 0.01
CEC ($cmol_c$ kg^{-1} soil)	10.1 ± 1.3
NH_4-extractable cations ($cmol_c$ kg^{-1} soil)	
Ca	3.11 ± 0.02
Mg	0.80 ± 0.01
K	0.32 ± 0.01
Zn	0.008 ± 0.001
Cd	0.0002 ± 0.0000

Values are averages ± standard deviations ($n=3$).

Meck KgaA, Darmstadt, Germany. The content of acetate was adjusted at 43.1 mg kg^{-1} soil for all treatments by Na-acetate, referring to the amount of acetate added to the soil with the treatment 87 mg U kg^{-1} soil. For each U treatment, the soil was watered to field capacity, mixed thoroughly and incubated for 3 days at 4 °C before their contact with plants. Soil samples were analysed after incubation.

The pH was measured in water and 1 M KCl at 1/5 soil/solution ratio, exchangeable Ca, Mg, K, Zn, Cd and U by 1 M NH_4-OAc pH 7, total U and P by borate fusion method, available P by Olsen method, cation exchange capacity (CEC) by silver thiourea method (Chhabra et al., 1975), and water soluble U and P obtained by centrifugal filtering extraction of the soil solution from soil equilibrated at field capacity.

2.4. Experimental phase

Mycorrhizal and nonmycorrhizal plants were then transplanted to 1-l pots filled with an equivalent of 0.9 kg dry soil of each U treatment prepared as described above. Soil moisture was adjusted to field capacity daily by watering with deionized water. The duration of this experimental phase was 4 weeks, in same greenhouse conditions of photoperiod, light intensity, temperature and humidity as in phase of mycorrhization. Three replicate pots were prepared for all 12 treatments: U applications (six levels) × mycorrhizal (+AM) and nonmycorrhizal (−AM) plants.

2.5. Harvest and assessment of variables

The experiment was harvested 4 weeks after transplanting to 1-l pots. Plants in each pot were cut at the soil surface and roots were extracted from soil by washing. A subsample of the root system was retained for measurement of root AM fungal colonization, and root and shoot dry weights were then determined after drying at 80 °C for 24 h. Mineral contents in roots and shoots were determined after calcination at 550 °C for 24 h, followed by ash dissolution with 1.5 ml 12 M HCl.

In all solutions, U, Zn and Cd were measured by inductively coupled plasma mass spectrometry (ICP-MS), Ca, Mg and K by atomic absorption spectroscopy, and P colorimetrically with the molybdate-ascorbic acid method (Watanabe and Olsen, 1965). Transfer factors (TF) were calculated for U as ratio of U concentration in plant tissues in mg kg^{-1} dry weight over U concentration in soil in mg kg^{-1} dry weight.

Roots of the subsamples were cut into 10 mm segments length, cleared in 10% KOH, and stained with 0.1% trypan blue for measurement of root AM fungal colonization (Kormanik and McGraw, 1982). Fifty randomly selected segments were examined under the microscope. The frequency of AM fungal colonization ($\%F$) was calculated as the percentage of root segments colonized by either hyphae, arbuscules or vesicles. In addition, the intensity of colonization ($\%I$), i.e. the abundance of hyphae, arbuscules and vesicles in each mycorrhizal root segment, was determined using the method outlined in Declerck et al. (1996).

2.6. Statistical analysis

Statistical analysis of data was performed by ANOVA with the statistical software STATISTICA for Windows (StatSoft, 2001). Mean values were compared by the Fisher's least significant difference (LSD) test, and significant differences were considered at $P<0.05$.

3. Results

3.1. Uranium in soil

The background level of U was low (1 mg kg^{-1} dry soil) resulting in small fraction of NH_4-extractable and very small fraction of water-soluble U (Table 2). The addition of ^{238}U resulted in increasing total U concentration, NH_4-extractable and water-soluble U forms in soil. The NH_4-extractable U forms represented between 13% and 20% of the total U contents in soil. For all U treatments, the water-soluble fraction was very small in the range 0.05–0.12% of the total U contents in soil.

3.2. Root AM fungal colonization

Plants inoculated with *G. intraradices* were highly colonized by the fungus and typical intraradical structures (hyphae, vesicles and arbuscules) were observed for both observation times (Table 3). The frequency and intensity of root AM fungal colonization were not significantly influenced by the levels of U

Table 2
pH, and total, NH_4-extractable and water-soluble U after 3 days of incubation of the soil with ^{238}U added at different levels

U added (mg kg^{-1} soil)	pH-H_2O	Total U (mg kg^{-1} soil)	NH_4-extractable U		Water soluble U	
			(mg kg^{-1} soil)	(% of the total)	(μg kg^{-1} soil)	(% of the total)
0	5.01	1.0 ± 0.1	0.02 ± 0.00	2.2	0.5 ± 0.01	0.05
5	5.01	5.8 ± 0.1	0.7 ± 0.02	12.6	3.8 ± 0.02	0.07
8	4.99	9.3 ± 0.8	1.6 ± 0.03	17.0	5.4 ± 0.1	0.06
20	5.06	21.1 ± 1.8	4.2 ± 0.11	19.8	11.5 ± 0.2	0.05
42	5.10	42.8 ± 0.2	8.0 ± 0.13	18.7	21.5 ± 0.5	0.12
87	5.12	88.0 ± 0.5	16.8 ± 0.43	19.1	43.2 ± 0.5	0.05

Values are averages ± standard errors ($n = 3$).

added in soil. No root colonization occurred in non-inoculated plants.

3.3. Dry matter production

The plant mycorrhization significantly increased the shoot dry weight whatever the U concentration in soil (Table 3). Shoot dry matter produced was 19–39% higher for +AM plants than for −AM plants. For root dry weight, significant differences between +AM and −AM plants were observed only for plants receiving 5 mg U kg^{-1} soil. The shoot/root ratios were 6–30% higher for +AM plants than for −AM plants. Uranium treatments did not significantly affect the root dry matter for both +AM and −AM plants and the shoot dry matter for −AM plants. Some significant differences between U treatments were only observed for shoot dry matter of +AM plants, but no general trend emerged towards an increase or decrease as U level increased in the soil. During the period of plant growth, no particular symptoms of toxicity induced by U treatments were observed.

3.4. Element contents

Increasing U concentration in soil significantly enhanced the U concentration in both roots and shoots of −AM and +AM plants (Fig. 1). The U concentrations were 60–360 times higher in roots than in shoots. Values of U transfer factor (TF) were very low in the

Table 3
Frequency (%F) and intensity (%I) of root AM fungal colonization, root and shoot dry weight and shoot/root ratio for nonmycorrhizal (−AM) and mycorrhizal (+AM) subterranean clover (*Trifolium subterraneum* L.) grown on soil with ^{238}U added at different levels

U added (mg kg^{-1} soil)	AM Trt	Root AM colonization[a]		Dry matter production		
		%F	%I	Shoots	Roots	Shoot/root
				(g per pot)		
Before U contamination	−AM	0	0			
	+AM	68	21			
At harvest						
0	−AM	0	0	1.17	0.75	1.56
	+AM	92	23	1.39	0.83	1.67
5	−AM	0	0	1.19	0.81	1.48
	+AM	91	21	1.65	1.05	1.57
8	−AM	0	0	1.17	0.83	1.40
	+AM	93	26	1.47	0.92	1.60
20	−AM	0	0	1.19	0.91	1.31
	+AM	89	32	1.62	1.04	1.56
42	−AM	0	0	1.32	0.99	1.33
	+AM	83	30	1.59	0.92	1.73
87	−AM	0	0	1.16	0.89	1.31
	+AM	85	32	1.45	0.98	1.48
$LSD_{0.05}$		9.2	14	0.17	0.23	0.30
*Analysis of variance**:						
Mycorrhizal		–	–	***	*	**
Uranium		NS	NS	*	NS	NS
Myc × U		–	–	NS	NS	NS

[a] One-way ANOVA performed on data of +AM roots; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, *NS* not significant.

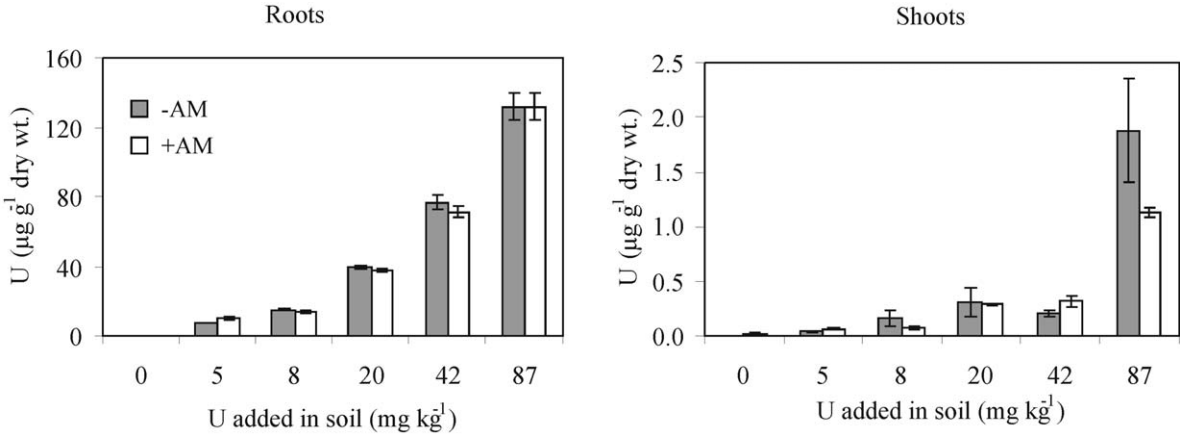

Fig. 1. Uranium concentrations in roots (left) and shoots (right) of nonmycorrhizal (−AM) and mycorrhizal (+AM) subterranean clover (*Trifolium subterraneum* L.) grown with ^{238}U added at different levels in soil. Averages and standard errors ($n=3$); $LSD_{0.05}$ of 0.42 and 10.7 for concentrations in shoots and roots, respectively.

range 8×10^{-3} to 2×10^{-2} for the shoots compared with values of 0.12–1.87 for the roots (Table 4). The AM fungus influenced U concentration only in shoots of plants grown at the highest level of U contamination, i.e. 87 mg U kg^{-1} soil. In this case, U concentration in shoots of −AM plants was 1.7 times as high as the concentration in +AM plants. No significant differences were observed between −AM and +AM plants for root U concentration, in all U treatments (Fig. 1).

Some significant differences were observed between U treatments in Ca, K, and Mg concentrations in plant tissues either for roots or shoots of −AM plants or of +AM plants (Fig. 2). Nonmycorrhizal plants grown at high U contents in soil had higher Ca concentration in roots and shoots than −AM plants grown at low U contents in soil, but this U treatment effect was not observed for +AM plants. The root Mg concentration was significantly higher for −AM and +AM plants grown with 42 and 87 mg U added per kg soil than for plants receiving less amounts of U. In general, the +AM plants had lower concentrations in Ca, Mg and K than the −AM plants, particularly for Mg in shoots. Phosphorus concentration tended to decrease with increasing U content in soil, particularly for roots of +AM plants, while it was markedly increased in the presence of the AM fungus (Fig. 2). It was 1.4 − 1.9 and 1.1 − 1.5 times higher in +AM plants than in −AM plants for roots and shoots, respectively. For Zn, the concentration was significantly lower for shoots of +AM plants than −AM plants in all U treatments, but no significant differences were observed for Zn concentration in roots between +AM and −AM plants (Fig. 3). Cadmium concentration significantly differed between +AM and −AM plants only for shoots of plants grown without U added in soil and with 42 mg U added per kg soil and for roots of plants grown with 42 and 87 mg U added per kg soil (Fig. 3). For these U treatments, plant mycorrhization enhanced Cd concentrations in roots and decreased it in shoots.

Table 4
Uranium transfer factors (TF, mg kg^{-1} shoot or root dry weight/mg kg^{-1} dry soil) for subterranean clover (*Trifolium subterraneum* L.) grown on two soils contaminated with different levels of U

U added (mg kg^{-1} soil)	AM Trt	TF	
		Roots	Shoots
0	−AMF	0.12	0.002
	+AMF	0.18	0.019
5	−AMF	1.27	0.007
	+AMF	1.79	0.011
8	−AMF	1.67	0.018
	+AMF	1.55	0.008
20	−AMF	1.87	0.015
	+AMF	1.78	0.014
42	−AMF	1.78	0.005
	+AMF	1.65	0.008
87	−AMF	1.50	0.021
	+AMF	1.50	0.013
$LSD_{0.05}$		0.42	0.015
*Analysis of variance**:			
Mycorrhizal		NS	NS
Uranium		***	NS
Myc × U		*	NS

* $P<0.05$, *** $P<0.001$, NS, not significant.

4. Discussion

In the present study the effects of the arbuscular mycorrhizal fungus *Glomus intraradices* on U uptake by clover were evaluated under varied concentrations of U in soil. Most of information on U uptake by plants was obtained from native plants growing on naturally U-contaminated soils (Ibrahim and Whicker, 1988; Saric

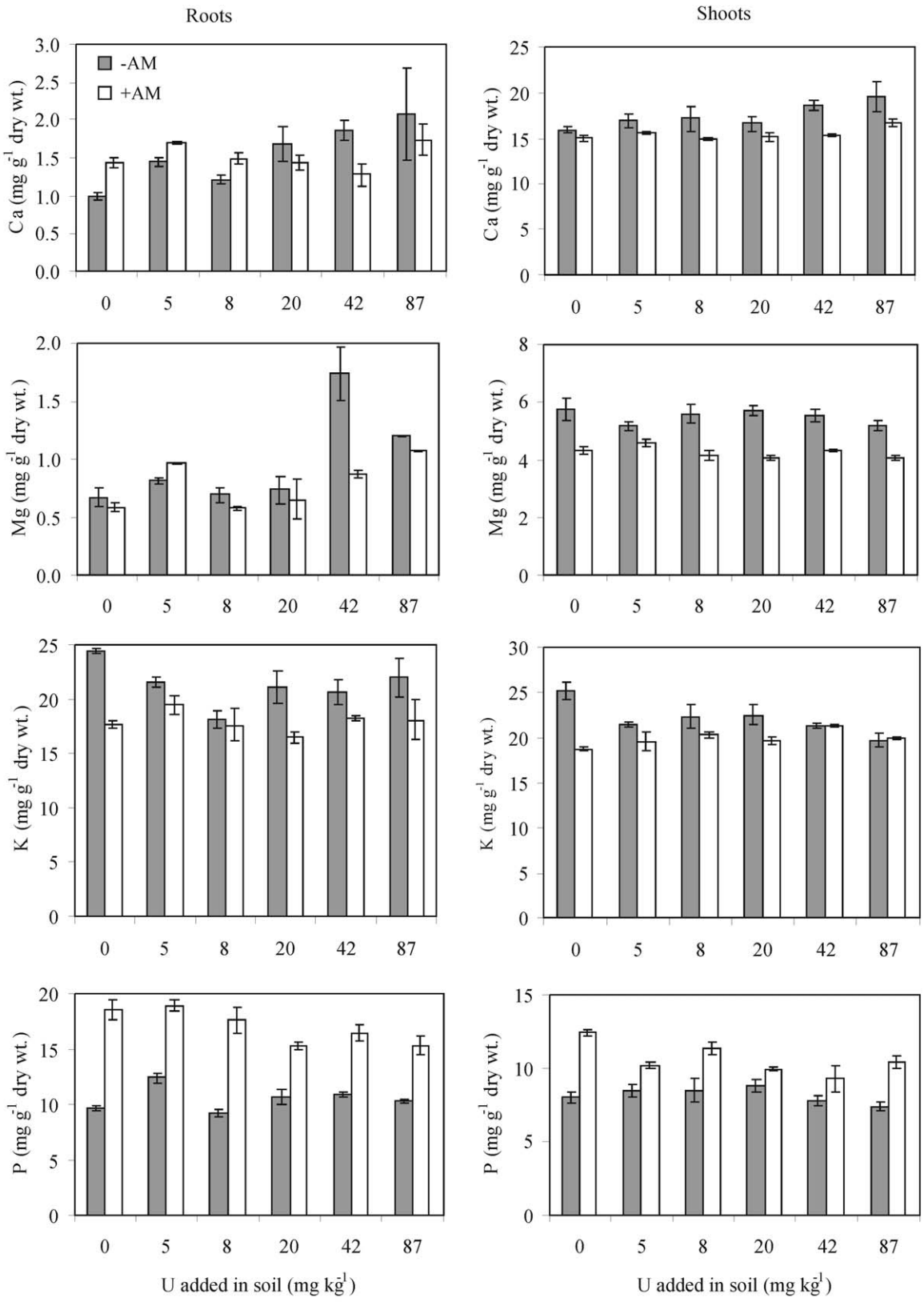

Fig. 2. Calcium, Mg, K and P concentrations in roots (left) and shoots (right) of nonmycorrhizal (−AM) and mycorrhizal (+AM) subterranean clover (*Trifolium subterraneum* L.) grown with ^{238}U added at different levels in soil. Averages and standard errors ($n=3$); $LSD_{0.05}$ of 2.2 for Ca, 0.59 for Mg, 2.1 for K and 0.45 for P concentration in shoots, and $LSD_{0.05}$ of 0.61 for Ca, 0.27 for Mg, 3.2 for K and 0.59 for P concentration in roots.

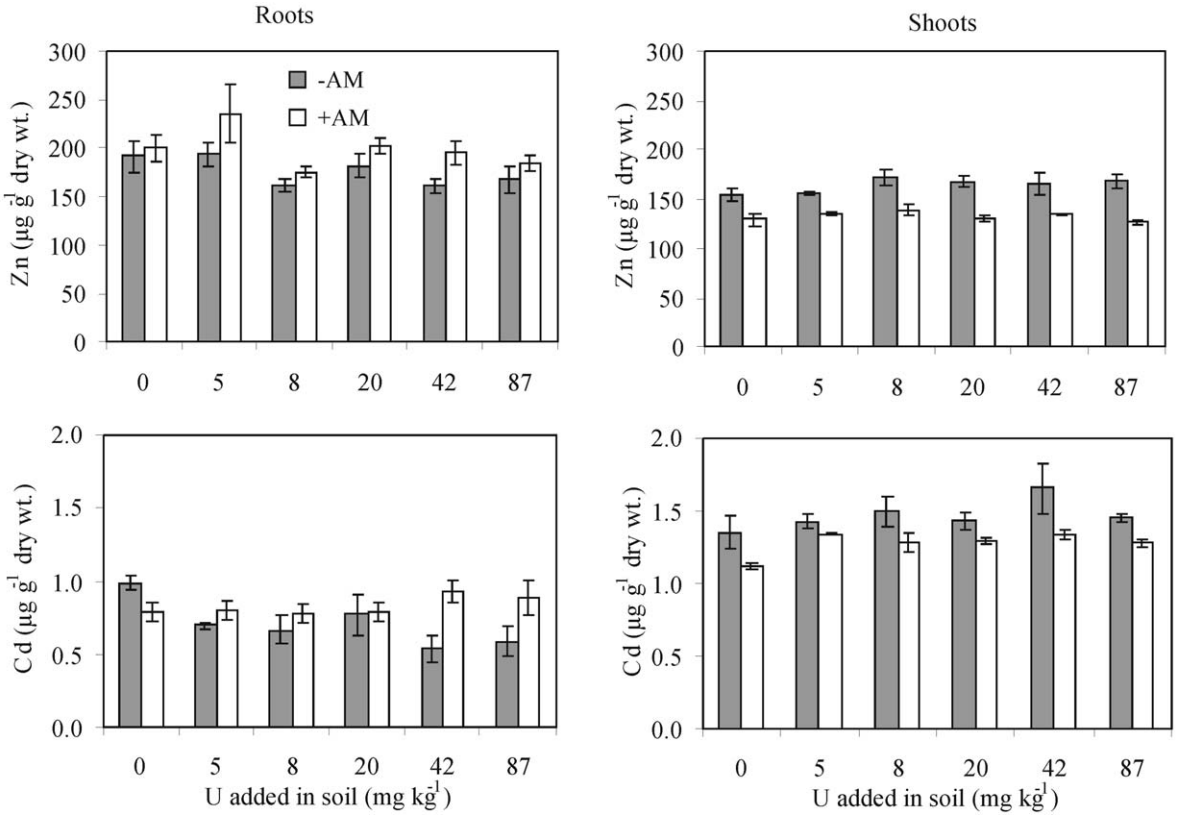

Fig. 3. Zinc and Cd concentrations in roots (left) and shoots (right) of nonmycorrhizal (−AM) and mycorrhizal (+AM) subterranean clover (*Trifolium subterraneum* L.) grown with ^{238}U added at different levels in soil. Averages and standard errors ($n=3$); $LSD_{0.05}$ of 16.6 for Zn and 0.22 for Cd concentration in shoots, and $LSD_{0.05}$ of 39.1 for Zn and 0.25 for Cd concentration in roots.

et al., 1995), and plants grown in pots filled with U-contaminated soil (Ebbs et al., 1998; Huang et al., 1998; Vandenhove and Van Hees, 2002) or plants grown in pots with U added in nutrient solution (Ebbs et al., 1998), but often without considering the effects of plant symbiotic microflora on U uptake.

Increasing U concentration in soil up to 87 mg kg^{-1} soil, or 90 times that of the U background, resulted in increased U concentration in roots and shoots, but it did not decrease the biomass production neither for the roots nor for the shoots. Similar results were reported by Sheppard et al. (1992) who detected no harmful effects on plant growth at U levels below 300 mg U kg^{-1} soil. In contrary, other studies reported that increasing U levels in soil from 1 to 6 mg kg^{-1}, close to the normal background ranges, resulted in a continuous decrease of wheat and tomato yields (Gulati et al., 1980). In these studies and in numerous other studies, the total U concentration in soil was considered in relation to U accumulation and U-related disorders caused to plants, without considering its availability for plants. However, as indicated by the results presented in Table 2, exchangeable forms may represent a small fraction of the total U content in soil, while the fraction representing the water-soluble forms may be extremely low. The absence of significant U effects on plant biomass production suggested that at all U levels tested in this study the U concentration in soil solution was too low to impair the growth of plants. The pH of the soil used in this study (around 5) was probably the key factor limiting the solubility of U added in soil, in accordance with the pH-dependency of U solubility (Grenthe et al., 1992).

High root AM fungal colonization with typical arbuscules, vesicles and hyphae observed for all inoculated plants, at all U levels in the soil, indicated that the tested concentrations of U contamination were not harmful to *G. intraradices*. The known beneficial effects of plant mycorrhization such as enhancement of biomass production, mostly attributed to improved mineral nutrition (Smith and Read, 1997) were confirmed by this study. Mycorrhizal plants produced higher shoot dry matter than nonmycorrhizal ones. This AM fungal effect appeared to be mainly due to the improvement of P uptake by the mycorrhizal fungus as the P concentration was higher in roots and shoots of mycorrhizal plants than in nonmycorrhizal plants. Numerous studies reported similar results on the enhancement of P concentration in plant tissues and this beneficial effect of plant mycorrhization was attributed to an active uptake of P from the soil and it translocation to plants by the extraradical mycelium of AM fungi (Clark and Zeto, 2002; Jakobsen et al., 2002; Smith and Read, 1997).

The concentration of macronutrient cations in plants were also influenced by the root AM fungal colonization. The presence of the AM fungus tended to decrease the K concentration particularly in roots as well as the Ca and Mg concentrations in shoots. No general trend was reported in the literature for the acquisition of these elements by AM plants in that increases, no effects, and decreases have been observed, as recently reviewed by Clark and Zeto (2002).

Uranium is a heavy metal and does not play any known role in plant nutrition. Like other heavy metals, U is tolerated in small quantities and results in toxicity when accumulated at high concentration in plants (Ebbs et al., 1998). The most novel information obtained from this study was the decrease of U concentration in shoots of only mycorrhizal plants grown at high level of U in soil. This suggested that the AM fungus *G. intraradices* has the potential to reduce U uptake by plants, especially by limiting its translocation from roots to shoots. This finding supported results from numerous studies reporting that mycorrhizal fungi often protected plants against high accumulation of toxic elements in the shoots, as it was reported for Al (Rufyikiri et al., 2000), Cd (Joner and Leyval, 1997), Zn (Burleigh et al., 2003; Li and Christie, 2001) and Pb (Malcova et al., 2003). The increase of metal retention in the roots to reduce metal concentration in shoots was hypothesized as the main mechanism involved, and the intraradical hyphae markedly contribute to this retention in the host roots. A high U concentration in intraradical fungal hyphae, of an undefined AM fungal species, than in the host root tissues was reported (Weiersbye et al., 1999), probably due to particular chemical conditions prevailing in the intraradical fungal cells. These conditions may include large P concentration (Nielsen et al., 2002; Pfeffer et al., 2001) and weakly acidic to neutral pH (Jolicoeur et al., 1998), promoting the formation of U-phosphate complexes and precipitates in the intraradical hyphae, and thus limiting its delivery to root cells.

Mechanisms related to physiological interactions between the fungus and host root cells may also be involved. At molecular scale, mycorrhizal colonization can regulate the expression of gene encoding plasma membrane transporters of elements and thereby affecting element accumulation by plants (Burleigh and Bechmann, 2002; Hildebrandt et al., 2002; Rosewarne et al., 1999). Burleigh et al. (2003) observed that the expression of Zn transporter gene (*MtZIP2*) was reduced in the roots of mycorrhizal *Medicago truncatula* Gaertn 'Jemalong' (line A17) at both moderate (1.2 mg kg^{-1} soil) and high (100 mg kg^{-1} soil) Zn fertilization and this down-regulation corresponded to a reduced concentration of Zn within the host plant tissues (roots and leaves) relative to uncolonized controls. Thus, these authors attributed the reduced Zn levels within mycorrhizal plants to a 'dilutive effect' caused by the growth response of colonized plants to numerous benefits of mycorrhization such as improved P nutrition. Such mycorrhiza-induced dilution probably also occurred for Zn and Cd in our experiment (Fig. 3), whereas the decreased shoot U concentration in mycorrhizal plants grown at the highest level of U in soil cannot be adequately explained as a dilution effect because shoot growth responses to mycorrhizas were similar at all U levels.

Although the mechanisms discussed above might have contributed in decreasing U concentration in shoots of mycorrhizal plants grown at high U level in soil, none of these assumptions allow to understand why at low concentrations, mycorrhizal and nonmycorrhizal shoots accumulated the same amount of U. Other factors such as the duration of the experiment (Entry et al., 1999) or the pot size (Leyval and Joner, 2001) may have concealed the mycorrhizal effects at low concentrations in soil.

In conclusion, this study on U and mycorrhizal fungal interactions gave novel information indicating that plant mycorrhization resulted in the decrease of U concentration in shoots of mycorrhizal plants grown at high level of U in soil. Mycorrhizal fungi could be potentially effective in protecting plants exposed to high levels of U in soil by limiting its accumulation in shoots. Other tests should be done to determine the role of other factors, particularly the effects of AM fungal species, duration of the experiment and soil types. The beneficial effects of the AM fungus observed in this study raise an interest to consider the role of AM fungi in plant-based strategies of remediation of highly U-contaminated soils.

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