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Arbuscular mycorrhizal fungi mediated uptake of ^{137}Cs in leek and ryegrass

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Abstract

In a first experiment of soil contaminated with ^{137}Cs, inoculation with a mixture of arbuscular mycorrhizae enhanced the uptake of ^{137}Cs by leek under greenhouse conditions, while no effect on the uptake by ryegrass was observed. The mycorrhizal infection frequency in leek was independent of whether the ^{137}Cs-contaminated soil was inoculated with mycorrhizal spores or not. The lack of mycorrhizae-mediated uptake of ^{137}Cs in ryegrass could be due to the high root density, which was about four times that of leek, or due to a less well functioning mycorrhizal symbiosis than of leek. In a second experiment, ryegrass was grown for a period of four cuts. Additions of fungi enhanced ^{137}Cs uptake of all harvests, improved dry weight production in the first cut, and also improved the mycorrhizal infection frequencies in the roots. No differences were obtained between the two fungal inoculums investigated with respect to biomass production or ^{137}Cs uptake, but root colonization differed. We conclude that, under certain circumstances, mycorrhizae affect plant uptake of ^{137}Cs. There may be a potential for selecting fungal strains that stimulate ^{137}Cs accumulation in crops. The use of ryegrass seems to be rather ineffective for remediation of ^{137}Cs-contaminated soil.

Keywords: Arbuscular mycorrhizae; AMF; Caesium; ^{137}Cs; Ryegrass; Leek; Radionuclides

1. Introduction

From the Chernobyl accident in 1986, many radionuclides were dispersed in different types of ecosystems. ^{137}Cs was the most important, due to abundance, long half-life (30 years) and chemical similarities to potassium. In terrestrial ecosystems, its entry into food chains, in the long term, is mainly determined by root uptake (Rosén et al., 1998). Therefore, processes affecting the root uptake of ^{137}Cs in arable ecosystems have been extensively investigated after the Chernobyl accident (Gray et al., 1996; Rosén et al., 1999; Ciuffo et al., 2002; Ehlken and Kirchner, 2002; Massas et al., 2002), as well as in

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forest ecosystems (Guilitte et al., 1994; Gillett and Crout, 2000).

Other studies have shown the importance of fungi on the ability to increase the uptake of radionuclides (Bakken and Olsen, 1990; Fulker et al., 1999; Steiner et al., 2002; Vinichuk and Johanson, 2003), and especially due to the fruiting bodies of ectomycorrhizae fungi (Haselwandter et al., 1988).

The fungal hyphae penetrate the soil around the plant roots effectively. As a result, the plant gains access to a larger volume of soil, which in turn leads to higher nutrient access in the soil. The benefits to plant production through increased uptake of phosphorus, as well as of manganese, molybdenium and zinc, have been shown (Tinker and Gildon, 1983; Gildon and Tinker, 1983; Jakobsen and Heidmann, 1989; Smith, 2000). Increased plant uptake of toxic metals, due to mycorrhiza, has also been reported (Li et al., 1991; Wang and Chao, 1992, Bürkert and Robson, 1994, Pawlowska et al., 2000).

Minor attention has been given to how arbuscular mycorrhizal fungi (AMF) affect the plant uptake of ^{137}Cs, despite the fact that most agricultural plant species form this type of mycorrhizae (Strandberg and Johansson, 1998; Staddon and Fitter, 2001). Results reported so far, however, are inconsistent. Some AMF species may enhance the uptake of ^{137}Cs (Drissner et al., 1998; Weiliang et al., 2003), whilst others appear to decrease it (Berreck and Haselwandter, 2001). Thus, the impact of AMF on plant uptake of ^{137}Cs needs further investigation. The aim of the present study was to investigate if AMF affect the plant uptake of ^{137}Cs, and if AMF can be of value to increase phytoremediation or removal ^{137}Cs from contaminated soils by growing ryegrass or leek.

2. Materials and methods

2.1. Soils

In autumn 1960, soil was collected from a field site in the county of Västergötland, longitude 13°25′ W and latitude 64°60′ N. The soil had been used in a long-term microplot experiment, following contamination with ^{137}Cs in 1961. Then, it was contaminated with 35.7 MBq/m^2, mixed homogeneously within the plough layer of soil, and crops were grown for many years. After 42 years, the activity had decreased to 13.9 MBq/m^2. The soil was used for cultivation of grass, cereals and peas during the 42-year period (Haak and Lönsjö, 1996). Uncontaminated soil was also collected from the same field site in 1960 and stored under cover, where it has been uncultivated for 42 years.

Both the contaminated soil grown and uncontaminated soil ungrown for this time period were used in pot experiments with leek and ryegrass to compare the influence of different crop history on mycorrhizae and its influence on root uptake of ^{137}Cs. In another pot experiment with ryegrass, only contaminated soil was used to compare the influence of two different inocula of mycorrhizae on root uptake of ^{137}Cs. Treatments without inoculation of mycorrhizae were used as control (Fig. 1).

Prior to use in these experiments, the soil, a loamy one, was sieved (2 mm) before use. Soil dry weight was determined after drying at 104 °C for 24 h. The soil is described in Table 1. Soil pH was determined with a 1:5 paste of soil and water. Total C, N and S were determined after dry combustion on a C, N, S autoanalyzer (LECO). Extractable K, Ca and P were determined by extracting (5 g of soil) sample with Al solution and HCl solution (Egnér et al., 1960). Spore numbers were determined by the wet sieving method (Raznikiewicz et al., 1994).

2.2. Mycorrhizae

Fungal inocula were prepared by inoculating seedlings of a plant mixture containing French marigold, tomatoes, meadow fescue, white clover and timothy with soil from a nearby field with a limited P-fertilization regime. The plants were grown in pots containing 250 g autoclaved expanded clay balls (Leca), soil added in a proportion of 1 part soil to 15 parts clay balls. One hundred spores were mixed into the top 2.5 cm layer of each pot. The spores were prepared as described by Raznikiewicz et al. (1994). The pots were kept in a greenhouse and supplied with sterile tap water when necessary. At the time of flowering of the white clover, the plants were allowed to wilt by ending the watering. The contents of the pots, i.e., fungal hyphae/spores/roots/sand, served as the mixed inoculant in the forthcoming pot experiments with ryegrass and leek. The pots were stored at 10 °C prior to use.

Experiment	^{137}Cs treated soil	Untreated soil (without ^{137}Cs)
With mycorrhiza		
(First exp.) Mixed inoculum	Leek	Leek
(First exp.) Mixed inoculum	Ryegrass	Ryegrass
(Second exp.) Inoculum I	Ryegrass, 4 cut	Ryegrass, 4 cut
(Second exp.) Inoculum II	Ryegrass, 4 cut	Ryegrass, 4 cut
Without mycorrhiza		
(First exp.)	Leek	Leek
(First exp.)	Ryegrass	Ryegrass
(Second exp.)	Ryegrass, 4 cut	Ryegrass, 4 cut

Fig. 1. Design of the two experiments.

Additional fungal inoculants, Inoculum I and II, respectively, for use together with ryegrass (Fig. 1), were prepared from single spore cultures from the ^{137}Cs-contaminated soil as follows. Single spore cultures were prepared by picking individual spores from the ^{137}Cs-contaminated soil after sieving and inoculating individual seedlings of sterile white clover with one spore each. The white clover seedlings were sterilized with hypochlorite and germinated in petri dishes (Somasegaran and Hoben, 1994). After germination, the white clover seedlings were placed in sterile plastic pipette tips containing sterile quartz sand and placed in a plastic rack filled with some sterile tap water in the bottom, allowing the pipette tips contact with the water. Two days after transferring, each clover plant was inoculated with a single spore of mycorrhizae, placed in a greenhouse for 4 weeks and, when necessary, supplied with sterile tap water.

Table 1
Mean values of soil characteristics for the loam soil used in the experiments

pH (H_2O)	6.0
C (%)	2.5
N (%)	0.2
S (%)	0.025
K–Al (mg/kg soil)	148
K–HCl (mg/kg soil)	1000
P–Al (mg/kg soil)	167
P–HCl (mg/kg soil)	900
Ca–Al (mg/kg soil)	2350
Bulk density (g/cm^3)	1.4
CEC (meq/kg soil)	118
Particle size (%)	
Sand	38.3
Silt	44.1
Clay	17.6
Mycorrhiza (spores per 100 g soil)	215
^{137}Cs (Bq/kg soil d.m.)	55,200

^{137}Cs content and mycorrhizal spores derives from the contaminated soil.

The clover plants within the pipette tips, were placed in previously prepared containers with Leca (heat-expanded clay spheres) balls where a mixture of various plant species consisting of tomatoes, corn, marigolds and meadow fescue were raised, the plants being 3 weeks of age when the white clover seedlings were introduced. After raising the mixed plant cultures as described above, the plants were allowed to wilt and the contents of the pots, i.e., fungal hyphae/spores/roots/sand Leca, were stored at 10 °C prior to inoculation.

2.3. Crops

Two species of plants were studied, leek (*Allium porrum*) and ryegrass (*Lolium perenne*). Two experiments were designed and are presented in Fig. 1. Four

replicates of each treatment with leek and three replicates with ryegrass were employed in the first experiment. In the second experiment with ryegrass, five replicates were used. The treatments consisted of ^{137}Cs-contaminated soil and the same soil uncontaminated, with or without adding inoculum prepared as described above at a level of 600 spores per pot. The seeds were placed on the soil surface, and covered with a layer of Leca. The pots used were cylindrical plastic containers with an inner diameter of 7.0 cm and 11.5 cm in height and contained 600 g dry weight (d.w.) of soil. Plants were watered when necessary with sterile tap water supplemented with a quarter strength Hoagland's solution every second week (Noggle and Fritz, 1976).

In the first experiment with leek and ryegrass, the soil was inoculated with mixed fungal inoculum, mixed inoculum (Fig. 1). Two weeks after germination of the seeds, plants in each pot were thinned to give 3 and 13 plants of leek and ryegrass, respectively. The leek plants were grown for 132 days, and when harvested, separated into shoots and roots. The ryegrass plants were harvested three times every 30 days after seeding. After the third harvest, the roots were separated from soil in a water bath. Above ground material was dried at 70 °C for 48 h, air dried for 24 h and weighed. Portions of the roots (less than 10% of the total root d.m.) were randomly excised and investigated for appearance of mycorrhizal infections (Raznikiewicz et al., 1994).

In the second experiment with ryegrass and ^{137}Cs-contaminated soil, free of mycorrhiza, the soil was inoculated with two separate fungi, Inoculum I and Inoculum II, respectively (Fig. 1). The two inocula used were selected at random from the 20 single fungal spore cultures, which had been raised. The experimental design included inoculation of ^{137}Cs-contaminated soil with two separate fungal strains and replicated five times. Growth conditions were the same as above. The inocula were placed on the seeds, to get an inoculum level equal to 600 spores per pot, and for the nonmycorrhizal treatments, sterilized heat-expanded clay balls were added instead. Plants were harvested at intervals at a plant age of 4, 10, 13 and 15 weeks.

2.4. Radiometry and statistical analysis

The ^{137}Cs specific activities in the soil samples and in the crops were determined using a calibrated HP Ge-detector. The ^{137}Cs specific activity was expressed as Bq/g d.w. All results were decay corrected to the date of sampling.

The results were subjected to analysis of variance and treated statistically according to SAS GLM edition 6.03 (SAS, 1988).

3. Results

3.1. Uptake of ^{137}Cs by leek

Uptake of ^{137}Cs by leek in the ^{137}Cs-contaminated soil with added mycorrhizae was higher, 2.23 Bq/g d.w. than in ^{137}Cs-contaminated soil without mycorrhizae, 1.56 Bq/g d.w. ($P<0.001$), although the mycorrhizal infection frequency was similar (Table 2). However, the biomass production of leek grown in either ^{137}Cs contaminated or uncontaminated soil did not differ ($P<0.05$). Inoculation with mixed strains of mycorrhizae improved the biomass production in the uncontaminated soil ($P<0.05$), which was initially

Table 2
Impact of mycorrhizal inoculation on ^{137}Cs specific activity, above ground biomass production, and infection frequency of roots in leek, ±standard deviation, $n=4$

Treatment	Contaminated soil			Uncontaminated soil	
	Specific activity (Bq/g)	Biomass d.w. (g)	Infection efficiency (% in roots)	Biomass d.w. (g)	Infection efficiency (% in roots)
Uninoculated	1.56	2.40	40.8	2.86	0
S.D.	±0.23	±0.28	±36.7	±0.33	
Inoculated	2.23	2.66	44.8	3.18	34.4
S.D.	±0.41	±0.12	±34.2	±0.28	±20.6

Table 3
Impact of mycorrhizal inoculation on specific activity of ^{137}Cs from three harvests, accumulated above ground biomass production from three harvests, and infection frequency of roots in ryegrass in the final harvest, ±standard deviation, n=3

Treatment	Contaminated soil			Uncontaminated soil	
	Specific activity (Bq/g)	Biomass d.w. (g)	Infection efficiency (% in roots)	Biomass d.w. (g)	Infection efficiency (% in roots)
Uninoculated	8.22	2.64	36.8	2.36	0
S.D.	±6.96	±1.10	±28.9	±0.19	
Inoculated	9.10	3.12	17.0	2.59	8.0
S.D.	±6.57	±1.04	±6.9	±0.25	±7.9

free of mycorrhizae. In the uncontaminated soil, inoculation also improved mycorrhizal infection (P<0.05).

3.2. Uptake of ^{137}Cs by ryegrass

In the first experiment with ryegrass grown under similar conditions to the leek, it was shown that both the ^{137}Cs specific activity and the biomass production were slightly increased in the contaminated soil (Table 3). The ^{137}Cs uptake by ryegrass was 8.22 Bq/g d.w. for uninoculated soil and 9.10 Bq/g d.w. for inoculated soil. The difference was not significant as shown from the accumulated figures of harvests and specific activities of ^{137}Cs presented in Table 3. The mycorrhizal infection frequency was twice as high in the uninoculated than in the inoculated soil. In the uncontaminated soil, which initially did not contain any mycorrhizae, adding mycorrhizae improved the mycorrhizal infection (P<0.05).

In the second experiment with the ^{137}Cs-contaminated soil, biomass production of ryegrass was considerably increased due to inoculation in the first cut (P<0.05), but not in the three following ones. The mycorrhizae infection in roots increased significantly, and was much higher for innoculum II than for innoculum I. (P<0.05; Table 4).

There was a large difference for ^{137}Cs specific activities (Bq/g) and in percent uptake of ^{137}Cs (of that of added to soil), between uninoculated (0.26–3.77 Bq/g) and inoculated (0.63–5.80 Bq/g) treatments. Arbuscular mycorrhizae (AMF) significantly enhanced the ^{137}Cs concentration both in Inoculum I and in Inoculum II. This was consistent in the ryegrass over a growth period of four cuts. The significance level was P<0.001 in the first three cuts and P<0.05 in the final cut (Table 5). The level of ^{137}Cs in root was high from 23.34 to 42.50 Bq/g d.w., for uninoculated and Inoculum II, respectively.

Table 6 shows the accumulated uptake of ^{137}Cs (cuts 1–4). Influence of inoculation is clearly seen, compared to that of the control. Both inocula are more pronounced for specific activities of ^{137}Cs than for uptake of ^{137}Cs, as the latter is also related to the yield of biomass. Of more interest was phytomerediation, or removal of radiocaesium by cropping (Table 6). As seen, mychorrhiza are effective in increasing uptake of ^{137}Cs (particularly in the roots) but the percent uptake of ^{137}Cs is very low. In Table 6, biomass yields

Table 4
Biomass production of ryegrass, g/pot, grown on ^{137}Cs-contaminated soil, uninoculated, and inoculated with two strains of arbuscular fungi, Inoculum I and Inoculum II

Treatment	Cut 1, 4 weeks	Cut 2, 7 weeks	Cut 3, 10 weeks	Cut 4, 15 weeks	Roots	Inf. %
Uninoculated	0.61	0.80	0.60	0.43	0.20	19.2
S.D.	±0.12	±0.05	±0.04	±0.09	±0.04	±3.9
Inoculum I	0.92	1.04	0.68	0.43	0.25	33.4
S.D.	±0.08	±0.10	±0.04	±0.13	±0.07	±5.3
Inoculum II	0.95	0.95	0.65	0.44	0.25	53.8
S.D.	±0.04	±0.12	±0.06	±0.10	±0.05	±4.7

Mycorrhizal infection frequency in roots (Inf. %), n=5.

Table 5
Specific activity and uptake of ^{137}Cs in four cuts of ryegrass and roots grown on ^{137}Cs-contaminated soil, uninoculated, and inoculated with two strains of arbuscular fungi, Inoculum I and Inoculum II, n=5

Treatment	Cut 1, 4 weeks		Cut 2, 7 weeks		Cut 3, 10 weeks		Cut 4, 15 weeks		Roots
	Specific activity (Bq/g)	Uptake (%)	Specific activity (Bq/g)	Uptake (%)	Specific activity (Bq/g)	Uptake (%)	Specific activity (Bq/g)	Uptake (%)	Specific activity (Bq/g)
Uninoculated S.D.	0.26±0.05	0.0005	2.01±0.49	0.0069	3.77±0.61	0.0069	2.32±0.36	0.0034	23.34±5.33
Inoculum I S.D.	0.63±0.17	0.0018	3.87±1.04	0.0108	5.24±1.12	0.0108	2.83±1.00	0.0037	39.06±4.90
Inoculum II S.D.	0.70±0.16	0.0020	4.20±0.64	0.0114	5.80±1.19	0.0114	4.07±0.94	0.0054	42.50±8.37

(accumulated) are also given, observed per pot and estimated for field conditions (kg/ha).

4. Discussion

Radiocaesium (^{134}Cs and ^{137}Cs) nuclides are hazardous elements, which cause long-term irradiation to biota and man. During the last 50 years, a substantial amount of these radionuclides have been introduced into the environment and they have caused worldwide contamination of large areas. An understanding of the transfer of radionuclides from soil to plants is therefore important in order to be able to safeguard food production. Many data on soil-to-plant transfer are available but limited attention has been given to the role of arbusucular mycorrhiza (AM), although the majority of agricultural plants are mycorrhized (Strandberg and Johansson, 1998; Bunzl et al., 2000; Berreck and Haselwandter, 2001).

Our results demonstrate that AM colonization can increase ^{137}Cs plant uptake, at least under greenhouse conditions. However, the results do not support the use of the particular mycorrhiza studied for use in phytoremediation. This is contrary to the findings by Berreck and Haselwandter (2001), who reported a decrease of ^{137}Cs in plants colonized by arbuscullar mycorrhiza. These authors suggested that their results were due to the fact that ^{137}Cs was sequestered by the extraradial fungal hyphae and not transferred to the plants to the same extent as non-AM roots. Accumulation of radiocaesium in fungal structures has, as our results also show, previously been demonstrated by Haselwandter et al. (1988). However, our results have shown that uptake of ^{137}Cs by the plants studied here differed (Tables 2, 3 and 4). This led to the conclusion that plant characteristics and related mycorrhizal interactions influence ^{137}Cs uptake from ^{137}Cs treated soil, an observation also supported by Rogers and Williams (1986) and Roca et al. (1997). Thus, the contradictory findings point to the fact that basic knowledge of potential uptake mechanisms is needed to facilitate the design of countermeasures to reduce the transfer of radionuclides into plants.

In our study of the ^{137}Cs-treated soil, inoculation with arbuscular mycorrhiza significantly increased uptake of ^{137}Cs by leek but not by ryegrass (Tables 2 and 3), which could be explained by the much higher root density for ryegrass than for leek. In a modeling study, Kirk and Staunton (1989) found that higher density of roots in the soil resulted in more ^{137}Cs being accumulated by a wide variety of grassland plants. Another explanation may relate to the mechanism by which mycorrhizae enhance uptake of nutrients

Table 6
Accumulated uptake of ^{137}Cs in grass cuts 1–4 in percent of that given to ^{137}Cs-contaminated soil, and biomass of grass (shoots) and roots, g d.w. per pot and estimated per hectare, for ryegrass uninoculated and inoculated with two strains of arbuscular fungi, Inoculum I and Inoculum II

Treatment	Uptake of ^{137}Cs in %		Yield, g/pot		Yield, kg/ha	
	Shoots cut 1–4	Roots	Shoots cut 1–4	Roots	Shoots cut 1–4	Roots
Uninoculated	0.015	0.014	2.44	0.20	7 630	630
Inoculum I	0.029	0.030	3.07	0.25	9 500	780
Inoculum II	0.031	0.032	2.99	0.25	9 340	780

through the extraradial hyphal network increasing contact with soil. If the root density is extremely high, then the presence of extraradial hyphae would be of little additional help for the plant, and these could instead be competitive in nutrient uptake, because extraradial hyphae may sequester part of the extractable ^{137}Cs in the soil. Inconsistent results of investigations between plant and fungal species with respect to ^{137}Cs uptake have also been reported. McGraw et al. (1979) found that AM colonization of *Paspalum notatum* by 2 out of 10 AM fungal species led to a twofold increase in ^{137}Cs-contaminated leaf tissue. Dighton and Terry (1996) determined the ^{137}Cs-activity uptake by *Festuca ovina* and *Trifolium repens* from labeled soil. In *F. ovina*, AM infection resulting from the use of nonsterilized soils as inoculum did not enhance plant growth. Shoots showed higher ^{137}Cs concentration than roots and, in similar experiments with heather, translocation of ^{137}Cs activity to shoots increased in the presence of AM. In the case of *T. repens*, AM plants took up less radiocaesium than non-AM plants. There appeared to be no increased translocation of ^{137}Cs to the shoots.

The results presented have suggested that selection of mycorrhiza from soils exposed to ^{137}Cs could increase ^{137}Cs uptake by ryegrass particularly into the roots (Table 4). The limited translocation of ^{137}Cs to plant shoots, however, seems to indicate that this would be a rather ineffective method for the remediation of ^{137}Cs-contaminated soil. Other selections of mycorrhiza may be more effectively translocating ^{137}Cs in these or other crops.

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