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Inoculation with arbuscular mycorrhizae does not improve ^{137}Cs uptake in crops grown in the Chernobyl region

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ABSTRACT

Methods for cleaning up radioactive contaminated soils are urgently needed. In this study we investigated whether the use of arbuscular mycorrhizal (AM) fungi can improve ^{137}Cs uptake by crops. Barley, cucumber, perennial ryegrass, and sunflower were inoculated with AM fungi and grown in low-level radionuclide contaminated soils in a field experiment 70 km southwest of Chernobyl, Ukraine, during two successive years (2009–2010). Roots of barley, cucumber and sunflower plants were slightly or moderately infected with AM fungus and root infection frequency was negatively or non-correlated with ^{137}Cs uptake by plants. Roots of ryegrass were moderately infected with AM fungus and infection frequency was moderately correlated with ^{137}Cs uptake by ryegrass. The application of AM fungi to soil *in situ* did not enhance radionuclide plant uptake or biomass. The responsiveness of host plants and AM fungus combination to ^{137}Cs uptake varied depending on the soil, although mycorrhization of soil in the field was conditional and did not facilitate the uptake of radiocesium. The total amount of ^{137}Cs uptake by plants growing on inoculated soil was equal to amounts in plant cultivated on non-inoculated soil. Thus, the use of AM fungi *in situ* for bioremediation of soil contaminated with a low concentration of ^{137}Cs could not be recommended.

1. Introduction

The Chernobyl accident in 1986 and the event in Fukushima Daiichi in Japan in 2011 resulted in contamination of agricultural land with radiocesium (^{137}Cs). As ^{137}Cs is long-lived and can stay in the environment for many years, it could continue to present a long-term problem for food, food production, and a threat to human health. Strategies aimed at remediating soils contaminated with low concentrations of radionuclides, especially ^{137}Cs have been developed (Entry et al., 1999; Dushenkov et al., 1999). One strategy is based on the ability of fungi to accumulate and retain ^{137}Cs. Ectomycorrhizal fungi can accumulate appreciable amounts of ^{137}Cs within both their sporocarps and belowground mycelium. The percentage of the total ^{137}Cs inventory of forest soil found in fungal mycelium of ectomycorrhizal fungi may range from 0.1 to 50% (Vinichuk and Johansson, 2003), and levels of ^{137}Cs found in sporocarps appear to be at least one order of magnitude higher than in fungal mycelium (Vinichuk et al., 2010).

Also the role of arbuscular mycorrhizal (AM) fungi in radionuclide-polluted soils and their effects on radiocesium uptake and transfer to plants have been studied with the possible idea to exploit the fungi for bioremediation purposes (Rogers and Williams, 1986; Entry et al., 1999; Berreck and Haselwandter, 2001; Joner et al. 2004; Rufyikiri et al., 2004; Rosén et al., 2005; Dubchak, 2010; Gyuricza et al., 2010). Reported results are inconsistent and either demonstrate AM fungi can facilitate, reduce, or have no effect on ^{137}Cs uptake by host plants. Thus, the actual role of arbuscular mycorrhiza in plant uptake of radiocesium, and the capacity of AM fungi to accumulate and transport radionuclides, is not fully understood and remains challenging, as the results from experimental studies are inconsistent, inconclusive, uncertain, and even controversial.

Certainly AM fungi are involved in phosphorus uptake, but they might be involved in cations uptake. Recent studies (Hammer et al., 2011) demonstrated that AM fungi can selectively take up such elements as K, which is an analogue for Cs.

Thus, the objective of this study was to investigate whether AM fungi application to soil under field conditions enhanced ^{137}Cs uptake by crops. The parameters for this study were the aboveground

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plant biomass, the ^{137}Cs concentrations ratios (CR), the infection frequency of inoculated plant roots, and the total ^{137}Cs activity taken up by plants. As a working hypothesis, the assumption was AM fungi could be applicable for the phytoremediation of soils contaminated with low concentrations of ^{137}Cs.

2. Materials and methods

2.1. Study sites

Experimental plots were established near the settlements of Yazhberen (51°14′55″ N, 29°09′25″ E, site I) and Khrystynivka (51°14′14″ N, 29°13′09″ E, site II) in Narodychi, Zhytomyr Region, Ukraine, about 70 km Southwest of Chernobyl and a few kilometres away from the contaminated zone (Zone-1). In 2009, the ground ^{137}Cs soil deposition (to the depth 0–20 cm) was 93.1 ± 8.34 at site I, and 832 ± 53 kBq m^{-2} at site II.

2.2. Experiment layout

A randomized block design was used, which comprised three treatments: AM+, F and C. In treatment AM+, soil was inoculated with AM fungus *Glomus mosseae* before the experiment and in treatment F, soil was treated with fungicide: treatment C was assigned as a control and did not receive any fungicide or inoculum. The treatment was the same on a given parcel for the two consecutive years. All treatments had four replicates. Two experimental sites 495 m^2 (11 m × 45 m) each were subdivided into blocks with 1 m borders between. All together there were 120 (3 treatments × 5 crops × 4 replicates × 2 sites) individual experimental plots having the sowing areas of 7.2 m^2 (2.4 m × 3 m) and the portion of the sown area that was sampled to avoid border effects of 2.8 m^2 (1.4 m × 2 m).

2.3. Soils

The soil at the two sites was classified as albeluvisols umbric with a sandy loam texture derived from sandy fluvio-glacial deposits (IUSS, 2007). For site I, the main soil characteristics were 3.35% clay content in soil, 38.1% silt, 58.5% sand, 5.2 ± 0.06 pH $_{H2O}$, and 1.9% organic matter. For site II, the main soil characteristics were 4.75% clay content in soil, 42.9% silt, 52.3% sand, 6.6 ± 0.06 pH $_{H2O}$, and 2.9% organic matter content. The concentrations (mg kg^{-1}) of elements in the soil (extracted with 0.1 M $BaCl_2$) were Site I: Ca 66.0 ± 24.0, Mg 8.2 ± 3.0, and K 43.0 ± 9.0; Site II: Ca 65.1 ± 5.9, Mg 6.3 ± 1.2, and K 14.3 ± 0.9. Chemical analyze of soil is performed according to standard method used at Swedish University of Agricultural Sciences (Laborationskompendium i Markkemi, 2008).

2.4. Experiment description

In treatment AM + commercial mycorrhizal inoculant *G. mosseae* (MTT Agrifood Research, Laukaa Finland) containing live arbuscular mycorrhizal fungi (5–10 AMF propagules/g inoculum) was applied to the soil at a rate recommended by the producer and in the proportion of 5% based on volume before seeding and thoroughly mixing throughout the upper 2–3 cm to protect the inoculant from sun and heat. In treatment F, the fungicide Benlate (du Pont de Nemours & Co. (Inc.), Wilmington, Delaware, USA) was applied at a rate, calculated from the recommended rates for application in the field (Sukarno et al., 1993), to give final concentrations in the soil of 125 mg kg^{-1}. The crops used in the experiment were barley (*Hordeum vulgare* L.), cucumber (*Cucumis sativus* L.), perennial ryegrass (*Lolium multiflorum* Lam.), sunflower (*Helianthus* spp.), and spring oilseed rape (*Brassica* spp.). Plants were seeded in rows by hand and weeded as required. No fertilization was used in order not to negatively affect the uptake intensity. The seeds we used were certified and obtained from Lantmännen SW Seed, Sweden. The density of sowing was standard for the area. The air temperature and precipitation according data from Ovruch meteorological station are presented in Fig. 1. No irrigation was applied throughout the growing season. After harvest, plants were air-dried to constant weight and aboveground parts divided into seeds and remaining parts. Seeds (grains) were sieved and the remaining parts were cut to <2 mm pieces. In addition to harvested aboveground parts, the roots of plants were dug out, washed in tap water, air-dried, and then analyzed for ^{137}Cs activity.

2.5. Quantification of AM fungal colonization

2.5.1. AM fungi colonization test

Approximately 25–50 g wet weight of roots (all together 64 samples from both sites, treatment AM+, 4 crops × 4 replicates × 2 sites × 2 years) was randomly picked from mycorrhizal plants barley, cucumber, perennial ryegrass, and sunflower and cold-stained according to Koske and Gemma (1989) in order to study the fungal root colonization. Prior to staining, roots were stored in a freezer at −40 °C. Then, roots were carefully rinsed in water and placed in process and embedding cassettes (Histolab Products, Västra Frölunda, Sweden). The roots were immersed in 20% potassium hydroxide for one day, washed in tap water, and acidified with 1% hydrochloric acid. After this, the roots were stained with 0.05% trypan blue in a 14:1:1 lactic acid:glycerol:water solution by volume for one day, and then rinsed in a destaining solution (14:1:1 lactic acid:glycerol:water) until no visual blue staining remained. For examination, the roots were spread onto glass slides and the appearance of fungal infections was visually recorded under a

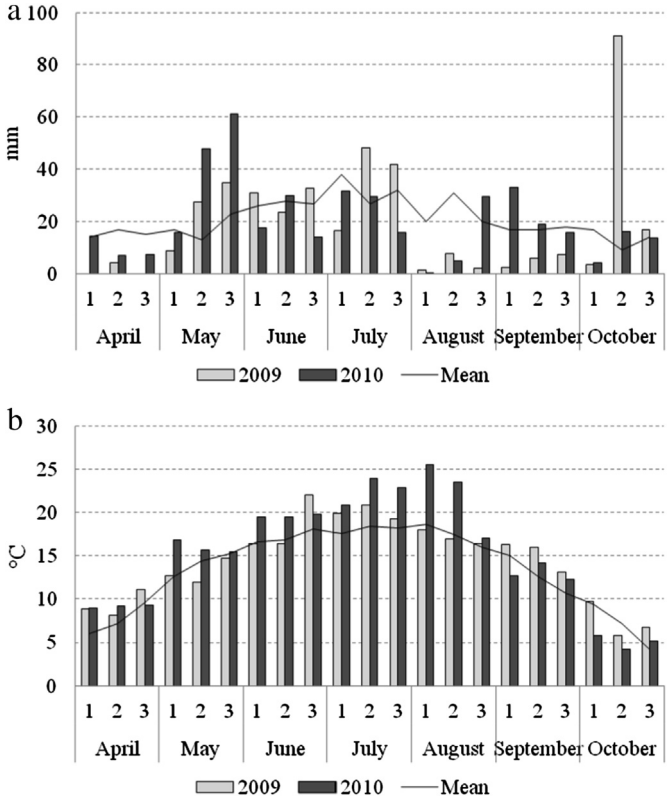

Fig. 1. The amount of rainfalling (a) and air temperature (b) at site I and site II.