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Role of arbuscular mycorrhizal fungi on the accumulation of radiocaesium by plants

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"The chief beauty of this book lies not so much in its literary style or in the extent and usefulness of the information it conveys, as its simple truthfulness. Its pages form the record of events that really happened. All that has been done is to colour them."

Jerome K. Jerome, preface to *Three Men in a Boat*

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I also would like to thank I. Jakobsen who invited me to his laboratory in Denmark. He provided me with a more intimate knowledge on AM transport and helped me throughout my work with his experience and friendship.

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I am also very grateful to L. Siragusa and P. Carlier for providing facilities, time and iunvaluable expertise for carrying out my ^{33}P, $^{134,137}Cs$ counting.

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List of Abbreviations

ABA: Abscisic acid

AM fungi: Arbuscular mycorrhizal fungi

AM-P *in vitro* culture system: Arbuscular mycorrhizal – plant *in vitro* culture system

CC: Culture compartment

Cs: Radiocaesium (or caesium if explicitely indicated)

EC: Experimental compartment

ECM fungi: Ectomycorrhizal fungi

ERM: Extraradical mycelium

HAM-P *in vitro* culture system: Half-closed arbuscular mycorrhizal – plant *in vitro* culture system

HC: Hyphal compartment

IRM: Intraradical mycelium

K: Potassium

KIR: Potassium inward rectifying channel

KOR: Potassium outward rectifying channel

KUP/HAK transporter: High affinity potassium transporter

MSR medium: Modified Srullu Romand medium

MSR^{Cs} medium: Adapted modified Srullu Romand medium for Cs transport studies

P: Phosphorus

RC: Root compartment

RHC: Root and hyphal compartment

ROC: Root-organ culture

SKOR: Stellar potassium outward rectifying channel

VIC channel: Voltage insensitive cation channel

%F: Frequency of root length colonized by arbuscular mycorrhizal fungi

%I: Intensity of root colonization by arbuscular mycorrhizal fungi

Glossary

Uptake by arbuscular mycorrhizal fungi: Uptake of ions by the extraradical mycelium of arbuscular mycorrhizal fungi.

Translocation by arbuscular mycorrhizal fungi: Movement of ions from the extraradical mycelium to the intraradical mycelium of arbuscular mycorrhizal fungi.

Transfer by arbuscular mycorrhizal fungi: Successive efflux of ions from the intraradical mycelium of arbuscular mycorrhizal into the root cell apoplasm and influx into root cells.

Transport by arbuscular mycorrhizal fungi: Consists of the successive uptake of ions by the extraradical mycelium of arbuscular mycorrhizal fungi, their translocation from the extraradical myeclium to the intraradical mycelium and their transfer from the extraredical mycelium to root cells.

Root to shoot translocation: Movement of ions from the roots to the shoot of plants.

Accumulation: Refers to the increased content of an element over time.

Author Contributions

The work presented here was performed solely during the course of this PhD.

The **Introduction, Context of the Study** and **Conclusions & Perspectives** of this thesis were written exclusively by myself. Some paragraphs of the **Context of the Study** are taken from a review article intended for a special issue of the Journal of Environmental Radioactivity. Note also, that the **Perspectives** are taken from the EC-project MYCOREMED to which I contributed markedly during the writing process.

The **Material & Methods** section is taken from a book chapter in which L. Voets and myself contributed for 20 and 80%, respectively.

My involvement in **Chapter I** was the collection and analyzes of the data. I also participated in writing the article that was published in Environmental Microbiology. As such, it was estimated that 40% of the experimental work, data analyzes and manuscript preparation were performed by myself.

The work of **Chapter II, III and IV** was carried out by myself from setting the goals to publication. Articles presented in **Chapter II** and **III** were respectively published in Environmental Pollution and

Environmental Microbiology. **Chapter IV** is planned to be published after additional experiments, but some of the results were already presented at the 5th International Conference On Mycorrhiza (ICOM5), Granada (Spain), on the 23-27 of July 2006.

The **Discussion** section is taken, in part, from a review article intended for publication in a special issue of the Journal of Environmental Radioactivity. This review was completely written by myself. It aimed at covering the major achievements of the EC-MYRRH project. I did not present here the results obtained on uranium (U). I, eventually, had to modify the original text to avoid references to points discussed in the part on U and to focus solely on Cs. Notice that two short reviews were also published in Radioprotection and in the book "In vitro culture of mycorrhizas" on the results of my experiments. None of them are presented here in order to avoid unnecessary repetions.

Remarks:

An article for which I was co-author (Voets *et al*, 2005) was not included in this thesis, even if my participation in its elaboration represented 30% of the work performed. Indeed, the system that was described and the results obtained using this system are presented in the Material & Methods section of this thesis.

The results obtained during the course of this PhD were presented at several conferences. Two posters were prepared and four oral presentations (three as speaker) were given during these events. An abstract for a conference that will take place after the achievement of this PhD was also accepted.

Finally, thanks to the experience gained during these five years of PhD, I participated in the writing of a European project (MYCOREMED 2006-2010) on the role of arbuscular mycorrhizal fungi on plant Cs accumulation, and a Belspo project (MYCARBIO 2006-2011) on the impact of these fungi on the biodiversity and C-balance in grassland ecosystems under climate change. Both projects were favorably evaluated.

INTRODUCTION

Numerous soils have been contaminated by radiocaesium (Cs) as a result of nuclear weapon testing and accidents at nuclear power-plant facilities. Management of the areas, which have been exposed to either intense or diffuse Cs pollution, has become a major environmental concern. Indeed, Cs pollution constitutes a serious risk for human health as Cs can enter the human body through the food chain. Because of the relative long half-life of its two isotopes (30.2 years for ^{137}Cs and 2.1 for ^{134}Cs) and chemical behavior similar to that of potassium (K), Cs is one of the most threatening radio-polluant present in the environment. In terrestrial ecosystems, this similarity between K and Cs implies an important risk of Cs contamination of the aboveground vegetation, as root uptake mechanisms appear closely related for these two elements.

Soil Cs contamination cannot be treated, in the majority of cases, by conventional physico-chemical remediation procedures due to the size of the areas contaminated and the prohibitive cost of this type of techniques in term of manpower, equipment and chemicals. To limit the accumulation of Cs in plants, agricultural-based countermeasures using mineral amendments have been performed, but their efficiency remains controversial.

Attractive alternatives, orientated towards the use of plants and microorganisms, or both in combination, have been proposed in the recent years as potential players in removing or render pollutants harmless. Indeed, it was determined that they could extract, sequester,

detoxify, transform, mobilize or immobilize various pollutants ranging from toxic elements to polycyclic aromatic hydrocarbons, heavy metals and radionuclides. Among these microorganisms, mycorrhizal fungi have received increased attention due to their recognized role in element transport and immobilization. Ectomycorrhizal (ECM) fungi were shown to accumulate high amount of Cs, but also to participate in plant Cs accumulation. It was therefore suspected that these fungi could play an important role in soil Cs remediation strategies.

However, if several researchers also suspected that the obligate arbuscular mycorrhizal (AM) fungal symbionts could participate in Cs remediation strategies, neither their involvement in Cs transport to their hosts nor their capacity to accumulate Cs was unambiguously demonstrated.

Studies on K transport and accumulation by AM fungi suggested that this element could probably be taken up by AM extraradical hyphae and concurrently be translocated with P. High concentrations of K were observed in intraradical hyphae and arbuscule trunk hyphae. It was suggested, that K probably plays the role of balancing cation for the predominant P forms and participates in hyphae turgor. It was also suspected that K could be transferred from fungal to roots cells to maintain osmosis. This suggested that Cs could possibly be transported by AM fungi, and that accumulation in the intraradical fungal mycelium could probably occur as well. However, no studies

clearly showed the involvement of AM fungi in plant Cs acquisition or accumulation.

CONTEXT OF THE STUDY

Radiocaesium

Radiocaesium does not occur naturally on earth, it is exclusively anthropogenic in origin

I- Radiocaesium in the environment

According to Coughtrey & Thorne (1983), the mean soil concentration of naturally occurring ^{133}Cs is between 0.3 and 25 $\mu g.g^{-1}$ dry soil. However, due to the absence of any biological role of this element, and to the fact that it is not toxic at these concentrations for plants and soil organisms, it received little attention until the middle of the 20th century. Starting from the 1940's, a growing environmental interest appeared following the release into the environment of its long-lived radioactive isotopes, ^{134}Cs (t1/2 = 2.1 years) and ^{137}Cs (t1/2 = 30.2 years), produced by the first nuclear fission reactions and aboveground nuclear weapon testing (ca. 3 – 7% fission yield). This interest, however, turned rapidly to a major and global concern when, in 1986, the fourth reactor of the Chernobyl nuclear facility burned (Bell *et al*., 1988). This catastrophe led indeed to the release of enormous quantities of many radionuclides into the environment and especially of radiocaesium (Cs). After the fallout of the nuclear cloud, Cs contaminated aquatic and terrestrial ecosystems world-wide, but the most exposed areas were situated in Europe (Avery, 1996). Notwithsanding that the concentrations of these radionuclides are

seven orders of magnitude lower than ^{133}Cs, even in highly contaminated sites (Livens & Rimmer, 1988), the major concerns are that they are high energy β/γ-emitters and can be taken up by aquatic and terrestrial organisms. As a consequence, Cs may threaten animal and human health due to its entry into the food chain by various trophic levels.

From the researches on the environmental fate of Cs, a general consensus emerged that Cs exhibits a biogeochemical behaviour rather similar to that of potassium (K). In terrestrial ecosystems, this implies that Cs display low mobility in the soil profile due to its adsorption on clay minerals (Cawse, 1983; Smith & Elder, 1999; Rosén *et al*., 1999; Delvaux *et al*., 2001) and a relative high one in biological systems (Carter, 1993). This low mobility in soil is problematic as Cs tends to persist in the upper soil layers, which are extensively explored by plant roots and soil microorganisms. Thus, in terrestrial ecosystems, the principal route of Cs entry into the food chain is the soil-to-plant pathway (Coughtrey & Thorne, 1983). The absorption of Cs by plants and its accumulation, therefore, represents the principal source of human exposure to these radionuclides (Shaw & Bell, 1994).

It was also noticed that soil fungi, including mycorrhizal fungi, could accumulate significant amount of Cs. These observations led to two major assumptions: (1) consumption of carpophores could participate in animal and human contamination, and (2) fungal

immobilization could be employed to stabilize Cs in contaminated soils.

However, if numerous studies have been performed on various groups of soil fungi, the role of AM fungi on Cs accumulation or transport to their host plants has been rather ignored (Declerck *et al*., 2001a).

II- Fungi as radiocaesium accumulators

Studies on the uptake of Cs by mycorrhizal fungi started after it was observed that high concentrations of Cs were recorded in European mushrooms (Grueter, 1971; Kalač, 2001). Comparisons between ectomycorrhizal (ECM) and saprotrophic species tended to suggest that ECM fungi have a very high capacity to concentrate Cs (Smith *et al*., 1993; Gillett & Crout, 2000) and could accumulate up to 270 times more Cs than plants growing in their immediate vicinity (Bakken & Olsen, 1990). Therefore, it was estimated that a large part of the Cs present in soils could be immobilized by fungal mycelia (Guillite *et al*., 1994). However, very few studies have analyzed the role of mycorrhizal fungi in the transport of Cs from soil to plants. To our knowledge, only two studies have been performed to determine if ECM fungi participate in the accumulation of Cs in plants (Brunner *et al*., 1996; Riesen & Brunner, 1996).

In these studies, it was shown that plants associated with ECM

fungi had lower root and shoot Cs contents compared to non-mycorrhizal plants. Therefore, it was suggested that because ECM fungi are able to accumulate Cs, they could reduce its availability to their host plants. However, Leyval *et al*. (unpublished data) demonstrated that two ECM fungi (*Paxillus involutus* and *Suillus bovinus* (Zn sensitive isolate)) were able to transport Cs to their hosts in a compartmented pot system. Cs concentrations ranged from 48 to 311 $Bq.g^{-1}$ in shoots and 147 to 406 $Bq.g^{-1}$ in roots of *Pinus sylvestris*. The same study also showed that *S. bovinus* (Zn tolerant isolate), *Laccaria laccata* and *Telephora terrestris* appeared unable to transport Cs. By comparing Cs root to shoot ratios in mycorrhizal and non-mycorrhizal plants, it was observed that Cs remained mainly in the roots of mycorrhizal plants. This suggested that the transfer of Cs by ECM fungi, when occurring, was probably low. In addition, it was also be suspected that root Cs uptake, trans-root Cs movements and/or Cs root to shoot translocation could be impaired by ECM fungal associations. Consequently, as ECM fungi could immobilize Cs and limit its accumulation in plants and especially in their shoot, they appeared to be good candidates for phytoremediation strategies orientated towards the stabilization of Cs in soils.

It has to be noticed that ECM fungi could also participate in the mobilization of Cs from clay minerals. Indeed, in a recent study, Yuan *et al*. (2004) suggested that protons released from ECM fungi could replace interlayer K of vermiculite and phlogopite minerals and that oxalate, also released by ECM fungi, could lead to biological

weathering. The capacity of AM fungi to participate in mineral weathering is still poorly studied. However, it appeared that they could participate in mineral solubilization by excreting proton and organic acids, but also by modifying soil microbial activity and biodiversity (Duponnois *et al*., 2004). This mobilization, which resulted in the uptake and accumulation of K by ECM fungi, is probably also valid for Cs, as K and Cs occupy the same sites of adsorption on clay minerals (Delvaux *et al.*, 2001). Finally, Thiry *et al.* (unpublished data) showed that following aboveground plant contamination, Cs present in plant tissues could be transferred to ECM fungi and accumulated in their fruit bodies. This result suggested that ECM fungi could participate in reducing plant Cs content and thus that ECM fungi are utterly relevant in understanding Cs bio-geochemical fluxes in terrestrial ecosystems. This observation also showed that there might be a time (seasonal) pattern in the flow of Cs between ECM fungi and their host. Indeed, if Cs can be transported by ECM fungi to their host, it might eventually be redirected to the fungi upon development of their fruit bodies. This could also be valid between soil and ECM fungi and therefore uptake rate of Cs by ECM fungi could be dependent on fruit body formation.

Phytoremediation

A ground-breaking technology

Over the last decades, numerous soils have been contaminated by radionuclides as a result of human activities such as mining, nuclear weapon production and testing, and nuclear accidents. Management of size-limited sites or larger areas, which have been exposed to either intense or diffuse radionuclide pollution, has become a major environmental concern.

The ultimate remediation of radionuclides is to remove and treat contaminated soils with various dispersing and chelating chemicals (Entry *et al*., 1996). This method can be envisaged for small-scale sites with high activity concentrations of radionuclides, even if cost in term of manpower, equipment and chemicals is rather prohibitive. However, for wider areas or sites with lower activity concentrations, it remains inapplicable (Zhu & Shaw, 2000). Furthermore, the increasingly strict legislative requirements for discharge of large volumes of often low-activity waste, for which the conventional treatment methods seem mostly ineffective or highly expensive, led the researchers to seek for other management strategies.

For instance, to limit the accumulation of radionuclides in plants, agricultural-based countermeasures have been suggested (see

Zhu & Shaw, 2000). Application of mineral or chemical amendments has been proposed to reduce the phytoavailability of radionuclides in soils (e.g. Lembrechts, 1993; Valcke *et al*., 1996; Vandenhove *et al*., 1996). However, these countermeasures methods were poorly reliable (Camps *et al*., 2003, Vidal *et al*., 2001; Zhu & Shaw, 2000).

Recently, attractive alternatives orientated towards the use of plants and microorganisms, or both in combination have been suggested. Indeed, some of these organisms have the capacity to remove pollutants or render them harmless (Zhu & Shaw, 2000; Barkay & Schaefe, 2001; Dushenkov, 2003; Eapen *et al*., 2003; Gentry *et al*., 2004; Kuiper *et al*., 2004; Suresh & Ravishankar, 2004; Lloyd & Renshaw, 2005). This strategy involves the use of the biological characteristics of plants and microorganisms to extract, sequester, detoxify, transform, mobilize or immobilize soil pollutants (i.e. ranging from toxic elements, polycyclic aromatic hydrocarbons, hevy metals to radionuclides).

Radionuclides remediation strategies using plants (i.e. phytoremediation) can be orientated towards phytoextraction when hyper-accumulation in aboveground organs is sought or phytostabilization when radionuclides can be immobilized in plant roots or in the soil (Dushenkov, 2003).

Microorganisms are of great interest for phytoremediation strategies. They can indeed participate in the mobilization or

immobilization of various pollutants including radionuclides (Gray, 1998; Steiner *et al*., 2002; Gadd, 2004). The combination of plants with rhizospheric microorganisms has been proposed, and efficient strategies have been developed in recent years (Gentry *et al*., 2004; Kuiper *et al*., 2004). However, the focus was mostly orientated towards the use of bacteria.

Since the 90's it has been recognized that mycorrhizal fungi could play a key role in soil remediation strategies of pollutants, including radionuclides (Entry *et al*., 1996; 1999). These symbiotic fungi occupy a unique situation at the soil-plant interface (Smith & Read, 1997; Chaplin *et al*., 2002). They develop an active *continuum* between an extraradical hyphal network spreading into the soil and an intraradical network developing within the roots of their host plants (Smith & Read, 1997). This *continuum* allows bi-directional exchange of carbohydrates from plant to fungus and minerals from fungus to plant (Ferrol *et al*., 2002). Such exchanges suggest a strong involvement of mycorrhizal fungi in carbon and various mineral elements cycling and influence on ecosystem processes (Rillig *et al*., 2004). The potential role of mycorrhizal fungi on radionuclides or other pollutants biogeochemical cycles was therefore suspected.

Arbuscular Mycorrhizal fungi

"The study of phycomycetous endotrophic mycorrhizas is therefore seen to have emerged as a reputable pursuit" (Harley, 1969)

After a century of poor consideration by the microbiologists, mycorrhizal fungi are now getting an incredible recognition as unique organisms, ubiquitous in terrestrial ecosystems, economically valuable and bestow upon exceptional properties. Indeed, if it took several decades to demonstrate that they can transport nutrients to their host, we currently know that they can also protect them from pathogens and osmostic stress, participate in soil structuration by producing glycoproteins, modify gene expression of their host, and affect plant community structure and ecosystem processes.

I- The performance of AM fungi in polluted environments

The majority of higher plants are associated with AM fungi. In the last decades, they were greeted with a considerable attention as it was noticed that they could participate in the acquisition of nutrients by plants and thus increase their growth. However, the involvement of AM fungi in the accumulation of pollutants in plants by transporting them to their hosts was also demonstrated (Joner & Leyval, 1997). In parallel, numerous studies have shown that plants can benefit from their symbiotic partners in polluted soils. Indeed, mycorrhizal fungi

can transform or immobilize pollutants in soils (Joner *et al*., 2000a; Meharg & Cairney, 2000; Jentschke & Goldbold, 2000; Ehlken & Kirchner, 2002; González-Chávez *et al*., 2004), therefore limiting their toxicity, or bioavailability and spreading into and from the soils. The purpose of this section is to illustrate what could be the mechanisms by which AM fungi could restrict or increase plant Cs acquisition and accumulation.

I-1- Avoidance and compartmentalization

Tolerance/sensibility of mycorrhizal fungi towards different pollutants has been widely studied (see for review Rapp & Jentschke, 1994; Jentschke & Godbold, 2000; Meharg, 2003 and Leyval *et al*., 1997; Hartley *et al*., 1997; Pawlowska & Charvat, 2004). Two principal strategies have been identified: avoidance and compartmentalization (Meharg, 2003).

Avoidance is a well-known strategy, which involves mechanisms that limit pollutants to enter an organism. It can be achieved for inorganic pollutants by (1) suppression of influx, (2) enhanced efflux, (3) release of complexing agents, (4) precipitation or binding onto cell outer surfaces and (5) release of agents that alter the pollutant speciation so that it cannot be taken up or is converted into non-toxic species. AM fungi inhabiting heavy metal-contaminated soils, for instance, are suspected to use this strategy (Pawlowska & Charvat, 2004), and it can be assumed that the main mechanism is

avoidance of uptake. Enhanced efflux is also likely (Nies, 1999) and experimental results pointed out that it is probably the mechanisms involved in Zn resistance of Zn resistant Suilloid fungi (Colpaert *et al*., 2005). Binding processes involving cell-wall components have been proposed for ECM, ericoid and AM fungi (e.g. Bradley *et al*., 1982; Joner *et al*., 2000a; Frey *et al*., 2000; González-Chávez *et al*., 2002; Bellion *et al*., 2006). Glomalin, a glycoprotein produced by AM fungal mycelium (Wright & Upadhyaya, 1996) could also be involved in heavy metal immobilization in the hyphosphere (González-Chávez *et al*., 2004).

Compartmentalization consists in the translocation of the pollutant taken up by the organism in an organ were it can be better tolerated or in a subcellular compartment where it can be stored away from the cytoplasm (Meharg, 2003). This strategy is yet scarcely demonstrated in mycorrhizal fungi with few data available. Some studies suggested that positively charged heavy metals and radionuclides could be complexed by vacuolar polyphosphates (Turnau *et al*., 1993; Tam, 1995; Hartley *et al*., 1997) as they are strongly negative polyanions, which use cations (K^+, Mg^+, Ca^{2+}) to balance their negative charges (Bücking *et al*., 1998). The ability of these polyphosphates to bind pollutants in mycorrhizal fungal mycelium and therefore to participate in their compartmentalization still needs to be clearly demonstrated, even if it has already been done for other microorganisms (Suzuki & Banfield, 2004). However, if pollutants may accumulate in the vacuoles containing these

polyphosphates, the function of these vacuoles is to allow translocation of P from the extraradical phase of mycorrhizal fungi to their intraradical one. Therefore, these vacuoles are considered as P carriers or P transitory storage structures (Callow *et al*., 1978; Cox *et al*., 1980; Ezawa *et al*., 2002; Viereck *et al*., 2004). Consequently, immobilization of pollutants by polyphosphates and compartmentalization within the motile vacuolar system of mycorrhizal fungi can only be considered as transitory. Indeed, when polyphosphates reach the intraradical mycelium and the sites of transfer, they are hydrolyzed to release P (Ezawa *et al*., 2002; Viereck *et al*., 2004). In this process, the cationic pollutants would be released within the fungal intraradical structures. This release of the pollutants at the symbiotic exchange sites might therefore facilitate their transfer towards the plant cells. However, their end destination might also be the intraradical vesicles for the AM fungi possessing these structures. Indeed, in these structures, Weiersbye *et al*. (1999) and Kaldorf *et al*. (1999) recorded high concentration of U, Mn, Cu, Fe, Ni and K. In these vesicles, but also in other specific organs such as storage structures, including fruit bodies, or auxiliary cells, accumulation of pollutants is also possible. For instance, heavy metals and Cs can accumulate in ECM fruit bodies and mycelium and their concentration can even reach unexpected high values (Byrne *et al*., 1976; Berthelsen *et al*., 1995; Nikolova *et al*., 1997; Krupa & Kozdroj, 2004).

Finally, it is important to notice that more than one mechanism of avoidance and compartimentalization may co-exist (Gadd, 1993;

Hartley *et al*., 1997; Leyval *et al*., 1997; Blaudez *et al*., 2000; Frey *et al*., 2000; Meharg, 2003).

I-2- Transport of pollutants

By extending beyond the depletion zone around the roots, and by enhancing their surface of exchange, AM fungi participate in the acquisition of diverse elements by plant (Smith & Read, 1997). Indeed, AM fungi develop an important extraradical mycelium, which maximize, at macro- and microscale levels, element uptake within heterogenous substrates.

As for roots (Barber, 1962), element uptake by AM fungi depends on both mass flow and diffusion. It is well known that AM fungi mostly provide to their hosts elements that are poorly mobile in soils (Smith & Read, 1997). It can therefore be assumed that, as AM fungi transport water (George *et al*., 1992), they can generate mass flow, so that these poorly mobile elements can be more easily taken up by AM hyphae (Tobar *et al*., 1994; Liu *et al*., 2002). However, if these elements are efficiently taken up by AM hyphae (e.g. P), mass flow may not be sufficient to replenish the hyphosphere of these elements. For instance, the contribution of K mass flow to the roots is usually very small due to efficient K root uptake (Kuchenbuch *et al*., 1986).

The capacity of AM fungi to transport nutrients and mineral pollutants has been widely investigated. Evidence has been given for the transport of P, N, Zn, Na, S, Cd, Se, Rb, Sr, Y and U, while for Be, Sc, Cr, Mn, Fe, Co, Zr, and Tc, it is likely that AM fungi can transport them, but in minute amounts (Marschner, 1995; Joner & Leyval, 1997; Suzuki *et al*., 2001; Chen *et al*., 2005). However, it was not shown yet whether AM fungi can transport K and Cs.

The demonstration that AM fungi can transport these elements was possible due to the development of compartmented systems in which roots and mycorrhizal fungi were allowed to develop in one compartment (i.e. the root compartment, RC) while the other compartment (i.e. the hyphal compartment, HC) was restricted to the exclusive development of the extraradical mycelium (ERM). Indeed, up to 1973, no proper experimental system was available to study nutrients or pollutants transport by AM fungi. Therefore, at the end of the 60's up to the beginning of the 70's, if numerous studies investigating the mechanisms behind the higher growth and P content of AM plants, it was not possible to determine whether AM fungi could transport P to their host (e.g. Daft & Nicolson, 1966; Sanders & Tinker, 1971; 1973 and Hayman & Mosse, 1972).

However, if these compartmented systems have led to striking results on element transport by AM fungi (see for references Marschner, 1995; Schweiger & Jakobsen, 2000), they presented some important drawbacks among which are the presence of undesirable

microorganisms, the difficulty to assay fungal growth and possible leakage of the element under study from the HC to the RC (Schweiger & Jakobsen, 2000; Rufyikiri *et al*., 2005). For these reasons, bi-compartmented *in vitro* systems became widely used for transport studies (Rufyikiri *et al*., 2005).

I-3- Effect of AM fungi on plant pollutant accumulation

The role of mycorrhizal fungi in plant pollutant accumulation could be linked to their influence on plant physiology and gene regulation. Indeed, plants also possess mechanisms to cope with soil contamination (see Marschner, 1995). Therefore, modification of plant physiology following mycorrhizal association could influence these mechanisms, plant tolerance/sensitivity to pollutants, but also the partition of the pollutants between roots and shoot. Such observations have already been done and add to delineate potential phytoremediation strategies using mycorrhizal fungi (see Leyval *et al*., 1997; Gaur & Adholeya, 2004). For instance, mycorrhizal fungal associations can alter the composition and amount of root exudates, which coud lead to modifications of element bioavailability directly, or indirectly by influencing the rhizospheric community (see reviews by Grayston *et al*., 1996; Landeweert *et al*., 2001; Jones *et al*., 2004). These modifications caused by mycorrhizal fungi might have a significant effect on pollutant bioavailability in the rhizosphere, but they remain, up to now, scarcely studied (but see Ahonen-Jonnarth *et al*., 2000; Vosatka, 2001; Martino *et al*., 2003). It should also be

noticed that exudates of mycorrhizal fungal hyphae could also influence pollutant bioavailability as they can release various compounds, including glomalin and organic acids (see above and review by Chalot & Brun, 1998; Jones *et al*., 2004) and modify microbial activity and composition (Andrade *et al*., 1997; Fillion *et al*., 1999; Marschner & Baumann, 2003). Observations by Rillig *et al*. (2006) even suggested that it could be possible to predict the hyphal community composition of associated microbes according to fungal phylogeny. However, no study has been performed to evaluate the role of the hyphospheric communities on pollutant bioavailability, but Wang *et al*. (2004) proposed that bacteria and actinomycetes in the hyphosphere of *Acaulospora lavis* could have participated in the degradation of di(2-ethylhexyl) phthalate (DEHP).

The complex relationship between host plants and mycorrhizal fungi requires a continuous exchange of signals that in turn regulate the mutual modification of metabolic and developmental processes during the course of the symbiotic association (Gianinazzi-Pearson, 1996). Phytohormones are probably involved in mycorrhizal symbiosis (Smith & Gianinazzi-Pearson, 1988; Schwab *et al*., 1991; Beyrle, 1995; Barker & Tagu, 2000; Ludwig-Müller, 2000; Harrisson, 2005). Modification of the levels of phytohormones and their balances are known to influence the root architecture and root to shoot biomass ratio (Ludwig-Müller, 2000; Torelli *et al*., 2000), photosynthate allocation and transformation (Blee & Anderson, 1998; Koch, 2004), for instance. Hypotheses that phytohormones may play a role in

pollutant tolerance has been suggested (Pan *et al*., 1989) and some authors (see Jentschke & Godbold, 2000) suspected that the modification of phytohormones balances or levels in mycorrhizal plants could participate to strengthen this tolerance. However, this is only supported by indirect and circumstantial evidences (Jones & Hutchinson, 1988; Hentschel *et al*., 1993; Jentschke *et al*., 1999; Cumming & Ning, 2003). This is principally due to the difficulty to demonstrate how phytohormones actually work (Beyrle, 1995), but also to the fact that not only the levels or balances of the phytohormones are to be taken into consideration. Indeed, the sensibility and regulation of the receptors are probably of great importance (Trewavas, 1981; 1991; Blee & Anderson, 1998). It is also to be noticed that mycorrhizal fungi produce hormones, but their role and function is not yet determined (Beyrle, 1995).

Regulation and induction of gene expression in both mycorrhizal fungi and their host is indispensable for recognition between the partners (Bécard *et al*., 2004; Breuninger & Requena, 2004), tuning of the bi-directional exchanges of nutrients and carbohydrates between them (Blee & Anderson, 2002; Burleigh & Bechmann, 2002; Ferrol *et al*., 2002; Requena *et al*., 2003) and could also affect the accumulation of pollutants in plants (Dupré de Boulois *et al*., 2005a; Göhre & Paszkowski, 2006). The control of gene expression depends on many factors. Phytohormones are probably involved as they are the mediator of various signals such as light, nutrient supply, biotic and abiotic stresses or the presence of other

organisms (e.g. Schmülling *et al*., 1997; Foster & Chua, 1999; Hoth *et al*., 2002; Bostock, 2005; Christmann *et al*., 2006). Among the genes that could be affected by mycorrhizal fungi, some plant nutrient transporter genes are included (Burleigh & Bechmann, 2002). A plasma membrane zinc (Zn) transporter from *Medicago truncatula* was down-regulated by AM colonization and was associated with a decreased concentration of Zn in the host roots and leaves (Burleigh *et al*., 2003). Furthermore, mycorrhizal plants showed no sign of Zn toxicity while the non-mycorrhizal plants developed signs of stunting. However, the authors, preferred to attribute the reduced Zn levels within the mycorrhizal plants to the dilution effect.

This dilution effect is a consequence of an improved mineral acquisition and growth of the plant due to the symbiotic association. However, it is also well known that variation of nutrient transport capacity and balance between nutritional improvement and carbon cost can lead to different plant growth improvement and even in some cases in a reduction of growth (Smith & Read, 1997). The association of different plant species or varieties with different mycorrhizal species or strains may indeed results in a wide array of responses, which can be enhanced or reduced by environmental conditions. Furthermore, AM fungi, in general, do not affect only the whole biomass of their host, but also their root to shoot biomass partition (Smith & Read, 1997). Therefore, the modifications of plant growth, root to shoot biomass partition and even the likely evolution of the mycorrhizal association from a reduced plant growth to an increased

biomass production should be carefully considered to clearly identify the role of AM fungi towards plant pollutant accumulation.

It is therefore crucial, to determine the influence of AM fungi on pollutant acquisition and accumulation by plants, to clearly identify the role of AM fungi on pollutant immobilization and transport, but also on their effects on their hosts.

II- AM fungi and radiocaesium

As mentioned by Kruyts & Delvaux (2002) for ECM fungi and Declerck *et al.* (2001a) for AM fungi, the potential role of mycorrhizal fungi in the transport of Cs to their host has been poorly documented. Even, for AM fungi, their capacity to take up Cs was not demonstrated. Mc Graw *et al*. (1979), Rogers & Williams (1986), Dighton & Terry (1996), Entry *et al*. (1999), Berrek & Haselwandter (2001), Rosén *et al*. (2005) have tried to determine whether AM fungi can transport Cs, but their efforts resulted in contradictory results on the role of AM fungi in plant Cs acquisition and accumulation. Indeed, by using mono-compartmented pot systems, in which either a mycorrhizal or non-mycorrhizal plant was growing, it was not possible to show transport as mention previously (see point II-2). Indeed, these authors could not differentiate the Cs that had been taken up by the plant roots from the one that could have been eventually transported or just translocated by the AM fungi. Furthermore, the determination of the capacity of AM fungi to

accumulate Cs could not be achieved, as collecting AM mycelium in soil and distinguishing it from that of other fungi is difficult.

In addition, in some of the studies previously performed, as best illustrated by the one of Joner *et al*. (2004), little or no consideration was given to the bioavailability of Cs, the concentration of K in the soil solution, or the K nutritional status of the plant, even if these parameters are probably highly relevant in determining whether AM fungi can transport and accumulate Cs.

III- Potassium, rubidium and caesium transport and accumulation by AM fungi

As it was not shown whether AM fungi can take up, translocate and transfer Cs to their host or accumulate it, information on the capacity of AM fungi to transport or accumulate stable caesium (^{133}Cs), K or Rb would have been most useful. Indeed, the chemical behavior of Cs is expected to be similar to that of ^{133}Cs, K and Rb, which all belong to group I (the alkali metals) of the periodic table. However, no report on ^{133}Cs transport or accumulation exists, and only one study reported that ^{86}Rb could be transported by AM fungi (Suzuki *et al*., 2001). The capacity of AM fungi to transport K is not demonstrated. Nonetheless, it was suggested that it would be reasonable to assume that AM fungi can transport K (Smith & Read, 1997). It should also be noticed that K was measured in the AM

mycelium, and that high concentrations were recorded in its intraradical phase (Ryan *et al*., 2003).

IV- Potential mechanisms of Cs uptake and efflux in plant and AM fungi

K transport mechanisms can be used to assess fungal Cs transport mechanisms as these mechanisms are probably similar. For plants, Cs transport mechanisms are known and have been already reviewed (White & Broadley, 2000; Zhu & Smolders, 2000).

K is an obligatory component of living cells. They consequently depend on its uptake, and eventually on its efflux, to ensure their growth and to keep themselves alive. K concentration in the cytoplasm is approximately 10^{-1} M, 3-5 orders of magnitude higher than in soils, where AM fungi and plant roots acquire it. This asymmetric distribution of K across the plasma membrane has been a constant during the evolution of plants and fungi, which colonized the emerged lands 450 millions years ago.

The K^+ transporters of plants are of three types, KUP/HAK, TRK and KIR, and in fungi, only KUP/HAK and TRK transporters appear to be present (Rodríguez-Navarro, 2000). Transporters of the KUP/HAK type seem to be present in all plants, but not in all fungi Rodríguez-Navarro (2000). In contrast, TRK type transporters are present in all the fungi and plants studied up to now (Rodríguez-

Navarro, 2000). However, Cs uptake models suggested that voltage independent cation (VIC) channels (also known as non-specific cation, NSC, channels) mediate most of the Cs^+ influx to root cells under 'single salt' conditions. It was also predicted that additional Cs^+ influx could also be catalyzed by KUP/HAK gene family, but that TRK and KIR do not participated in Cs influx in root cells. For mycorrhizal fungi, K^+ uptake could be mediated by TRK transporters. Indeed, Corratgé *et al*. (2006) recently identified a K^+ transporter, homologous to the yeast TRK transporter in *Hebeloma cylindrosporum*, *HcTRK*. This transporter could mediate K^+ influx, however its actual participation in K or Cs transport was not assayed. It has also to be noticed that Yoshida & Muramatsu (1998) and Baeza *et al.* (2005) suggested that the mechanism of Cs^+ uptake in fungi could be different from that of K^+.

In plants and fungi, the efflux of K^+ is operated through outward-rectifying K^+ (KOR) channels (Rodríguez-Navarro, 2000). KOR channels, such as stellar (SKOR) channels, dominate the loading of K^+ into the xylem, and are also permeable to Cs^+ (Gaymard *et al*., 1998). In mycorrhizal fungi, Corratgé *et al*. (2006), identified in *H. cylindrosporum* an ion channel from the Shaker family of voltage-dependent K^+ channels, *HcSCK*. This channel shares similarity to the KOR channels and Corratgé *et al*. (2006) found that it could mediate K^+ efflux. This channel could therefore be involved in K^+ transfer from ECM fungi to plants. However, Corratgé *et al*. (2006) did not perform Cs^+ efflux studies, and thus capacity of this channel to

participate in Cs transfer was not demonstrated. In AM fungi, no K^+ transporter or channel has been identified yet.

Finally, it should be noticed that at K soil concentration below 1mM, a K depletion zone can be created around the roots due to K uptake and the slow diffusion of K (Smolders *et al*., 1996; Waegeneers *et al.*, 2001; Waegeneers *et al*., 2005). As Cs concentration at the soil-root interface is poorly affected as compared to its concentration in the soil solution, the ratio of K/Cs decreased in the rhizosphere (Smolders *et al*., 1996; Waegeneers *et al*., 2001). The consequence of this lower K/Cs ratio on the capacity of roots to take up Cs by roots was modelled and revealved that Cs could be taken up at a higher rate than the one expected if the K and Cs concentration in the soil solution were taken into account (Waegeneers *et al*., 2005) and thus confirmed the supposition of Delvaux *et al*. (2000) and the observations of Gommers (2001). For AM fungi the creation of this K depletion zone at the level of the hyphosphere could also be possible if AM fungi could take up K.

It was also shown that a slight decrease of the soil water content, could dramatically reduce K concentration in the soil solution (Waegeneers *et al*., 2005). This results in a reduced diffusion of K that first lead to a strong reduction of the K concentration in the rhizosphere that cannot be compensate by mass flow (Kuchenbuch *et al*., 1986), and in turn to a higher capacity of the roots to take up of Cs (Waegeneers *et al*., 2005). It is possible that this phenomenon could

also occur at the level of the hyphosphere and thus increase the capacity of AM hyphae to take up Cs. However, it would only be possible if AM fungi could take up K at a sufficient rate so that mass flow and diffusion cannot replenish K in the hyphosphere.

RESEARCH OBJECTIVE

The role of arbuscular mycorrhizal (AM) fungi on the acquisition of radiocaesium (Cs) by plants remains poorly understood and is controvertial. The lack of clear results on the capacity of AM fungi to accumulate or transport Cs could be principally attributed to inadequate experimental systems used in previous studies. Also, the substrates, concentration of potassium (K) in the soil solution, plant K nutritional status could have interfered with the capacity of AM fungi to accumulate or transport Cs. Furthermore, the various AM fungi and plants tested could also explain the controversial conclusions obtained as AM fungi and plants have probably different capacity to accumulate and transport Cs. As a consequence, it was successively suspected that AM fungi could accumulate Cs in their extraradical (ERM) or intra-radical (IRM) mycelium, or transport Cs to their hosts.

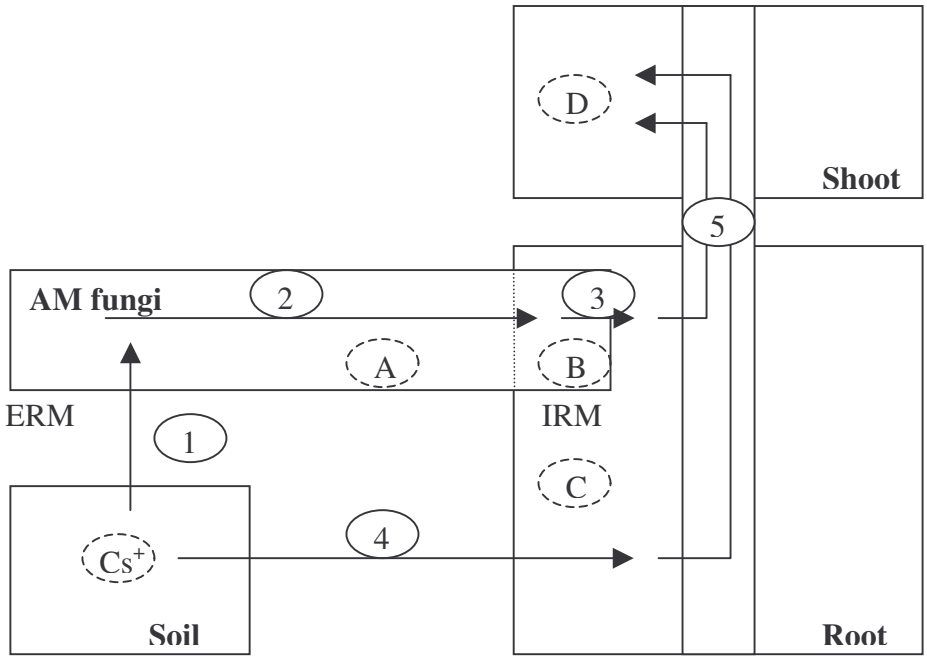
The objective of this work was therefore to identify whether AM fungi could accumulate Cs or transport Cs to their hosts.

This objective will be answered by the determination of (see Fig.1):

- The capacity of AM fungi to take up (1), translocate (2) and transfer (3) Cs to their host,
- The capacity of AM fungi to accumulate Cs in their ERM (A) and IRM (B),
- The relative contribution of AM fungi and roots in plant Cs acquisition by comparing the capacity of AM fungi to take up (1) and translocate (2) Cs with root Cs uptake (4),

- The influence of AM fungi on root (C) and shoot (D) Cs accumulation following Cs transport by AM fungi (1, 2 & 3) and root uptake (4),
- The influence of AM fungi on root to shoot translocation (5),
- The influence of potassium and phosphorus on the transport (1, 2 and 3) of Cs by AM fungi and on the accumulation of Cs in root (C) and shoot (D).

Fig. 1 Schematic representation of the mechanisms by which arbuscular mycorrhizal plants could acquire and accumulate radiocaesium.



1 Uptake of Cs by the extraradical hyphae of AM fungi.

2 Translocation of Cs from the extraradical mycelium (ERM) to the intraradical mycelium (IRM).

3 Transfer of Cs from the IRM to the root cells.

4 Cs root uptake.

5 Root to shoot Cs translocation following transport (1, 2 & 3) of Cs by AM fungi or Cs root uptake (4).

A Accumulation of Cs in the ERM following uptake (1) of Cs by AM fungi.

B Accumulation of Cs in the IRM following uptake (1) and translocation (2) of Cs by AM fungi.

C Accumulation of Cs following transport (1, 2 & 3) of Cs by AM fungi and/or Cs root uptake (4).

D Accumulation of Cs in the shoot following root to shoot Cs translocation (5).

OUTLINES OF THE THESIS

In this document, the major results obtained during the course of this PhD are presented as follows.

In the **Material & Methods** section, the two major experimental systems used for the experiments are described in details. In the **Chapter I to IV**, further precisions are given on how they were used.

In Chapter I, we investigated whether AM fungi can take up, translocate and/or accumulate Cs. Under root-organ culture (ROC) conditions, it was clearly demonstrated that AM fungi can take up and translocate Cs. In addition, accumulation of Cs was shown in the extraradical mycelium, but was probably over-estimated due to experimental constrains. The results of this experiment were published in Environmental Microbiology.

In **Chapter II**, we investigated the relative contribution of AM hyphae and roots, mycorrhizal or not, in the uptake, translocation and accumulation of Cs. Indeed, if in **Chapter I**, it was shown that AM fungi can take up, translocate and possibly accumulate Cs, it was not possible to determine whether it had any relevance as compared to the capacity of the roots to take up, translocate and accumulate Cs. The results showed that the uptake capacity of roots was higher than the one of AM hyhae. However, while roots did not translocate efficiently Cs, AM hyphae translocated most of it after its uptake. It was thus shown that the AM hyphae had a better capacity to translocate Cs. It should, nevertheless, be noticed that Cs translocation in the roots

could have been limited due to the lack of a sink, i.e. a shoot. At the same time, it was observed that AM colonization influenced the translocation of Cs in roots. Thus, it was suggested that AM fungi could restrict Cs loading into the xylem. This work was published in Environmental Pollution.

In **Chapter III**, we investigated the capacity of AM fungi to transfer Cs to their host plants. An *in vitro* culture system, in which an AM fungus was associated to an autotrophic plant, was used. The results of this work showed that AM fungi could take up, translocate and transfer Cs to their hosts. It was also observed that most of the Cs translocated to the AM plants remained in the roots, thus that Cs accumulated either in the fungal intraradical mycelium or in the root cells. These results were published in Environmental Microbiology.

In **Chapter IV**, we investigated the influence of K and P on the transport of Cs by AM fungi. In the previous chapters, it was suggested that K could reduce Cs transport by AM fungi, while P could increase it. However, the results showed that the influence of these elements was limited. It was nonetheless suspected that, by improving of the experimental design, a better assessment of the effect of these elements on Cs transport by AM fungi could be obtained. Therefore, the results of this study will be included in a larger work, in which the influence of K and P can be studied more precisely.

In the **Discussion,** the major results obtained are summarized and further commented. This section was taken from a review article intended for a special issue of the Journal of Environmental Radioactivity. This work also offers an early assessment on the potential role of AM fungi in phytoremediation strategies of Cs polluted soils.

Finally, the **Conclusions & Perspectives** are given. This section aims to pinpoint the major outcomes of this work and to present possible avenues to continue research on the role of AM fungi on Cs accumulation by plants.

MATERIALS AND METHODS

In vitro culture systems to study transport of elements by AM fungi

This work will be published in:

Mycorrhiza Manual, second ed., Varma (Ed.). Springer-Verlag, Heildelberg.

In vitro compartmented systems to study transport in arbuscular mycorrhizal symbiosis

Dupré de Boulois, H., Voets, L., Declerck, S.

Introduction

At the end of the 60's up to the beginning of the 70's, numerous studies investigated the mechanisms behind the higher growth and P content of arbuscular mycorrhizal (AM) plants (e.g. Daft & Nicolson, 1966; Sanders & Tinker, 1971, 1973 and Hayman & Mosse, 1972). However, the essential role of AM fungi in the uptake of P and its subsequent translocation and transfer to their host plants awaited the studies of Hattingh *et al*., (1973), Pearson & Tinker (1975), Rhodes & Gerdemann (1975) and Cooper & Tinker (1978, 1981) to be convincingly demonstrated. This major finding was obtained using ingenious bi-compartmented pot culture systems and isotopic tracers. In these systems, roots and mycorrhizal fungi were allowed to develop in one compartment (i.e. the root compartment, RC) while the other

compartment (i.e. the hyphal compartment, HC) was restricted to the exclusive development of the extraradical mycelium (ERM). Over the years, numerous other compartmented pot systems based on the same concept were developed to study AM fungal transport (see for instance Ames *et al*., 1983; Frey & Schuepp, 1992; Mäder *et al*., 1993; Schweiger & Jakobsen, 2000; Jansa *et al*., 2003; Smith *et al*., 2003; Tanaka & Yano, 2005).

However, if these compartmented pot systems have led to striking results on element transport by mycorrhizal fungi (see for references Marschner, 1995; Schweiger & Jakobsen, 2000), they presented some important drawbacks: (1) the presence of undesirable micro-organisms which could influence element bio-availability or transport processes, (2) the difficulty to visualize the development of the two partners of the symbiosis and to recover roots and extraradical fungal mycelium, (3) the possible interaction between the element and the soil matrix, (4) the potential direct uptake by the roots of the element under study due to tracer leakage towards the root compartment caused by diffusion or mass-flow, and (5) the complexity to perform physiological and molecular studies on extraradical fungal mycelium and mycorrhizal roots (Rufyikiri *et al*., 2005; Schweiger & Jakobsen, 2000). For these reasons, compartmented *in vitro* culture systems became widely used (Rufyikiri *et al*., 2005).

The aim of this chapter is to provide methodologies to perform

transport studies using bi-compartmented *in vitro* culture systems, with root-organs or whole plants as host, and to give a detailed description of the advantages and disadvantages of these systems.

Equipment and laboratory material

Equipment

- Horizontal or vertical laminar flow hood;
- Stereo-microscope (magnification x10 - x40);
- Bunsen burner or bead sterilizer;
- Temperature-controlled incubator;
- Climatic chamber;
- Rotating agitator;
- Thermo-fusible glue applicator.

Laboratory material

- Bi-compartmented Petri plates (usual diameter: 90mm);
- Polypropylene centrifugation tubes (50ml) ;
- Scalpels and forceps;
- Set of cork-borers ;
- Needles;
- Manual or motorized pipettors adapted to 5 to 20ml sterile pipettes;
- 1µl – 1000µl micropipettes with corresponding sterile tips;

- Cellophane wrap (roll of 2cm-large) and Parafilm^{TM} (note that Seal View^{TM}, Petri Seal^{TM} or other products can also be used);
- Thermo-fusible glue;
- Silicon grease;
- Black plastic bags (12x12cm);
- Inclined (0 to 45°) support for Petri plates.

Remark: All manipulations of plant, AM fungi, medium and isotopic tracers should be conducted under sterile conditions (i.e. under a laminar flow hood) and with sterile or sterilized laboratory material. Equipment and laboratory material that have no direct contact with the AM cultures do not require to be placed in a sterile environment or to be sterile or sterilized.

Culture media

Composition

In Table 1, the composition of the modified Strullu-Romand (MSR – Declerck *et al*., 1998, modified from Strullu & Romand, 1986) medium and the minimum (M) medium (Bécard & Fortin, 1988) is given. These media are the most widely used (see Table 1 in Cranenbrouck *et al*., 2005) for the *in vitro* culture of AM fungi.

Table 1 Mineral and vitamin composition of the modified Strullu-Romand (MSR) medium and minimal (M) medium.

	MSR medium	**M medium**
$N(NO_3^-)$ (µM)	3800	3200
$N(NH_4^+)$ (µM)	180	-
P (µM)	30	30
K (µM)	1650	1735
Ca (µM)	1520	1200
Mg (µM)	3000	3000
S (µM)	3013	3000
Cl (µM)	870	870
Na (µM)	20	20
Fe (µM)	20	20
Mn (µM)	11	30
Zn (µM)	1	9
B (µM)	30	24
I (µM)	-	4.5
Mo (µM)	0.22	0.01
Cu (µM)	0.96	0.96
Ca Panthotenate (µM)	1.88	-
Biotin (µM)	0.004	-
Pyridoxine (µM)	4.38	0.49
Thiamine (µM)	2.96	0.3
Cyanocobalamine (µM)	0.29	-
Nicotinic acid (µM)	8.10	4
Glycine (mg/l)	-	3
Myo-inositol (mg/l)	-	50

Stock solutions

The preparation of the MSR and M medium requires the preparation of stock solutions (see Table 2).

Table 2 Concentration of stock solutions ($g.l^{-1}$) to prepare the MSR and M medium.

		MSR medium	M medium
Macroelements			
	KNO_3	7.6	8
	KCl	6.5	6.5
	KH_2PO_4	0.41	0.48
	$MgSO_4.7H_2O$	73.9	73.1
Calcium Nitrate	$Ca(NO_3)_2.4H_2O$	35.9	28.8
NaFeEDTA		1.6	1.6
Microelements			
	$MnSO_4.4H_2O$	2.45	6
	$ZnSO_4.7H_2O$	0.28	2.65
	H_3BO_3	1.85	1.5
	$CuSO_4.5H_2O$	0.22	0.130
	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.034	-
	$Na_2MoO_4.2H_2O$	0.0024	0.002
	KI	-	0.75
Vitamins			
	Ca panthotenate	0.18	-
	Biotine	$0.18\ 10^{-3}$	-
	Pyridoxine	0.18	0.1
	Thiamine	0.2	0.1
	Cyanocobalamine	0.08	-
	Nicotinic acid	0.2	0.5
	Glycine	-	3
	Myo-inositol	-_	50

Preparation of culture media

For 1l of MSR or M medium:

- Mix together:
  - 10ml of the macroelement stock solution
  - 10 ml of the calcium nitrate stock solution
  - 5 ml of the NaFeEDTA stock solution
  - 1 ml of the microelement stock solution

- 5 ml of vitamin stock solution to prepare MSR medium or 1 ml for M medium
- Add 10g of sucrose
- After dissolution of sucrose, adjust the volume to 1l
- Adjust the pH to 5.5 with NaOH or KOH and HCl
- Add 3g.l^{-1} Gelgro^{TM} (ICN Biochemicals, Cleveland, OH), 5g.l^{-1} Phytagel^{TM} or 8g.l^{-1} agar.
- Autoclave for 15min. at 121°C under 1 bar pressure
- After autoclaving, the medium can be directly used but can also be stored for a few hours at 40-60°C.

Remark: Gelling agents may contain diverse macro-, micro- and trace elements. It can therefore be critical to determine their presence and concentration. E.g. MSR medium solidified by Gel-Gro^{TM} contains actually 4.38mM K and not 1.65mM (see Table 1), therefore the difference (i.e. 2.73mM K) is coming from the gelling agent.

Transport Studies with root-organ cultures (ROC)

While Hattingh *et al*., (1973) demonstrated for the first time that AM fungi can transport P, Mosse & Hepper (1975) reported the first *in vitro* co-culture between a root-organ and an AM fungus. However, in the mid 70's, this finding did not receive much consideration. Indeed, AM monoxenic cultures awaited the end of the 80's and the beginning of the 90's to be used and developed (Bago & Cano, 2005) when (1) the experimental conditions for AM monoxenic cultivation

were reformulated by the use of transformed root-organs to allow the cultures to be easily maintained (Bécard & Fortin, 1988), and (2) bi-compartmented Petri plates were used to allow the separation of the ERM from the mycorrhizal roots (St-Arnaud *et al*., 1995, 1996). These improvements significantly contributed to the success of this cultivation system (Bago & Cano, 2005) and in particular for transport studies (Rufyikiri *et al*., 2005).

At present, most of the monoxenic cultures of AM fungi are maintained on Ri T-DNA transformed root-organs as hosts and, in particular, on Ri T-DNA transformed carrot (*Daucus carota* L.) roots (see Table 1 in Cranenbrouck *et al*., 2005). These transformed roots offer the advantage to present a greater growth potential than non-transformed roots in culture media with limited supply of nutrients (Fortin *et al*., 2002). In addition, AM fungi in association with Ri T-DNA transformed roots show greater AM intraradical colonization and sustain higher ERM development than non-transformed root-organs (Cranenbrouck *et al*., 2005).

Virtually any AM fungal strain can be cultured under *in vitro* conditions on root-organs. However, for some species, continuous cultures are still complicated, explaining why a limited set of strains (e.g. *G. intraradices*, DAOM 197198, MUCL 41833; *G. proliferum,* MUCL 41827, *Glomus* sp., MUCL 43195) have been used for *in vitro* transport studies.

Preparation of ROC to perform transport studies comprises 3

major steps:

- Preparation of the Petri plates, i.e. adding medium in the RC and HC;
- Selection of the root;
- Inoculation of the root with AM fungal propagules.

Adding medium to the Petri plates

In Figure 1 and 2, different methods to add the medium in the bi-compartmented Petri plates are presented. In the RC, solid medium is added, while in the HC, either solid or liquid medium can be used. For each of the different methods, it is recommended to add the medium in the RC at least to the level of the top of the partition wall in order to facilitate the ERM spreading from the RC into the HC (Fig 1a, b and c and Fig 2a, b, c and d). Identically, the level of medium in the HC should also be high enough to help the ERM cross the partition wall. However, if liquid medium is added in the HC (Fig 1a, b and c), manipulations may lead to spilling of medium to the RC, so the level of medium in the HC should be cautiously chosen (typically between 5-20ml for a 90mm diameter Petri plate). If solid medium is used in the HC (Fig 2a, b, c and d), the level can be as high as in the RC (see Fig 2a and b).

Figure 1 Bi-compartmented Petri plates for pulse-chase transport studies with solid medium in the RC and liquid medium in the HC. The medium in the RC (a) reaches the top of the partition wall, (b) extends above the partition wall (i.e. convex meniscus) thanks to surface tensions, (c) is added in the entire Petri plate and extends 1-3mm above the partition wall; after solidification, the medium in the HC is removed using a scalpel and spatula (Dupré de Boulois *et al*., 2005b, adapted from Rufyikiri *et al*., 2003). In the HC, liquid medium is added and its level can be adapted to facilitate hyphal crossing or to restrict the possibility of spilling in the RC.

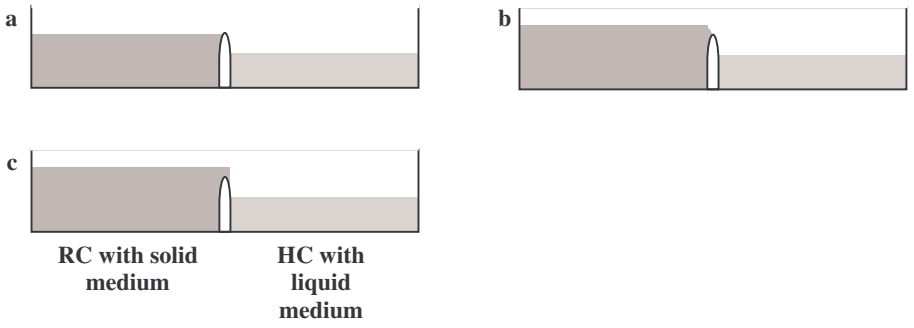
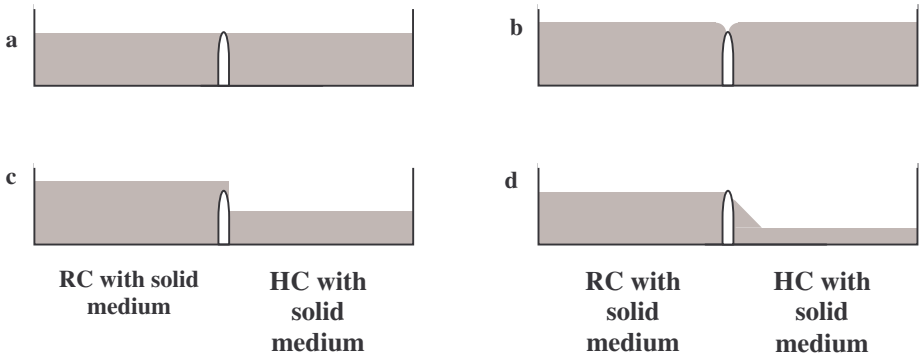


Figure 2 Bi-compartmented Petri plates for pulse-chase transport studies with solid medium in both the RC and HC. The medium in the RC can be added as described in Figure 1. (a) The medium in the HC reaches the top of the partition wall. (b) The medium in the HC extends above the partition wall (i.e. convex meniscus) thanks to surface tensions. (c) Solid medium is added in the entire Petri plate and extends 1-3mm above the partition wall, after solidification, the medium in the HC is removed using a scalpel and spatula and new solid medium is added to the HC (Dupré de Boulois *et al*., 2005b, adapted from Rufyikiri *et al*., 2003). (d) System of St-Arnaud *et al*. (1996), where a slope of medium against the partition wall is made by inclining the Petri plate at 45° using a support for Petri plates. Medium is then added in the HC so that it reaches the top of the partition wall. When the medium is solid, the Petri plate is placed horizontally and medium is added in the rest of HC.



Logically, the type of medium (i.e. MSR or M) used in the RC should be identical in the HC. However, it is advised not to add sucrose in the HC. Indeed, if no strict comparison has been made yet on the ERM growing with or without sucrose in the HC, it was suggested that the lack of sucrose could increase ERM and spore production (St-Arnaud *et al*., 1996). The absence of gelling agent in the medium of the HC can be justified as it enables an easy manipulation of the environment surrounding the ERM (e.g. modification of element composition, pH), and an easy access and harvest (Bago & Cano, 2005). However, if using liquid medium in the HC is extremely useful for pulse-chase experiments, it should be noticed that morphology of the ERM may differ from the solid medium (Bago & Cano, 2005). For instance, the regular pattern of development, with runner hyphae radically extending from the fungal colony, and producing BAS at regular intervals, might be impaired in liquid medium, in which runner hyphae are predominant and BAS are scanty and appear much less branched. These morphological differences should be taken into account as they may reveal other cytological or functional differences that may be important in the study of AM fungal transport. These modifications could influence (1) cell wall structure for adsorption/immobilization, (2) transport proteins distribution and abundance for uptake, and (3) cytoskeleton organization for translocation. However, if it is still unknown whether these modifications could impact transport by AM fungi, their consequences are probably minimal as several studies indicate that the symbiosis is functioning normally in nutrient and C transport (Pfeffer

et al., 2004 and references therein).

Selection of a Root-Organ for association

Following medium addition to the Petri plates, root-organs need to be placed in the center of the RC. This could be achieved with:

- A host root (60-100mm long, presenting secondary roots and/or secondary root primordia);
- A mycorrhizal root apex (20-40mm).

Remarks:

Root-organ selection: Root choice is essential for the successful establishment of the AM fungal symbiosis (Fortin *et al*., 2002). Ideally, a Ri T-DNA transformed carrot root should be 2 weeks old and present a vigorous taproot and a pyramidal pattern of lateral root development. Avoid selection of:

- Yellow or yellowing roots
- Roots exceeding 1mm in diameter
- Roots presenting swellings (especially at the level of the meristem) or deformations
- Roots presenting grey-translucent tips
- Roots presenting lateral roots close to the primary root tip (<2cm)
- Roots growing on the surface of the medium
- Punctured or broken roots

Mycorrhizal root-organ selection: If mycorrhizal root apexes are chosen it is important to verify the presence of ERM attached to the surface under stereomicroscope.

Notice that we do not recommend the transfer of a section of medium containing root apexes and ERM onto the RC to perform continuous culture of AM fungi (Cranenbrouck *et al*., 2005). Indeed, by doing so, the concentration of the elements present in the RC will be modified.

Inoculation of the root with AM fungal propagules

Fungal propagules should be inoculated onto the lateral roots and their primordia as well as in their direct vicinity, at a distance generally not exceeding 5mm.

At present, only *Glomus* species producing an extensive ERM bearing several thousand spores under ROC conditions have been used to perform transport studies (e.g. *G. intraradices*, DAOM 197198, MUCL 41833; *G. proliferum,* MUCL 41827, *Glomus* sp., MUCL 43195).

The isolation and inoculation of AM fungal spores from *Glomus* species producing several thousand spores are achieved as follow:

1. Extract a section of medium containing the number of spores desired from a well-established culture (3-6 months old culture) and transfer it to an empty Petri plate;
2. Add more or less twice the volume of filter sterilized citrate buffer (pH 6, 10mM: 1.8mM citric acid + 8.2mM sodium citrate);
3. Agitate the Petri plate slowly on a rotating agitator (50 rotation.min^{-1}) at 25-27°C until dissolution of the medium;
4. Transfer the spores to a new Petri plate containing sterile water using a 1000µL micropipette;
5. Separate the spores attached to the extraradical hyphae with needles in order to have clusters containing approximately 5-10 spores per cluster;
6. Inoculate the host root apexes or branched roots with approximately 100 spores per plate using a 200ml micropipette (try to minimize the volume of water);
7. Leave the Petri plate open to evaporate excess water (± 5 min);
8. Seal the Petri plate.

After inoculation, the Petri plates are incubated in a temperature-controlled incubator at 25-27°C in the dark. If the root-organs used have a negative geotropism, e.g. Ri T-DNA transformed carrot roots of the clone DC1, Petri plates should be placed in inverted position. It is advised to check each Petri plate every week during the first month to verify that no roots are growing into the HC. When roots are

crossing the partition wall, they should be trimmed and removed from the HC. This can be easily achieved with forceps and scalpel under laminar flow.

When the extraradical hyphae start to cross the partition wall (typically 2-4 weeks after initiation of the cultures), the Petri plate can be reverted and MSR or M medium (liquid or solid) lacking sucrose filled into the HC (see section 1.1). Isotopic labeling can then be initiated (see section 3).

Transport studies with autotrophic plants

The bi-compartmented ROC systems have been successfully used for numerous transport studies over the past 15 years as previously mentioned, but suffered from several major drawbacks. These can be summarized as follow: (1) the absence of photosynthetic tissues which prevent the determination of element transfer from AM fungi to root cells (except if using autoradiography or NMR (see Nielsen *et al*., 2002 and Pfeffer *et al*., 2004)) and photosynthate transport to the AM fungi, (2) the incomplete source-sink relationships between AM fungi and host, and root and shoot which could influence bi-directional transfers at the AM fungal-host interface and (3) the presence of sucrose in the culture medium and the lack of a normal hormonal balance which could alter plant-fungal symbiotic interactions including bi-directional exchanges (Fortin *et al*., 2002).

As previously mentioned, the first bi-compartmented systems to study element transport by AM fungi were developed in the early 1970's. These systems used, in particular, compartmented Petri plates where host roots grew in one compartment along with an AM fungus and where in the other compartment only extraradical fungal hyphae were allowed to develop. In these systems, sterility could only be maintained at the beginning of the experiment. However, as a small hole was cut in the lid of the Petri plate and the aerial part of the host was pulled through it, the plates could not be kept sterile (see for instance Pearson & Tinker, 1975). In an attempt to obtain both an autotrophic plant and AM fungi under *in vitro* conditions, numerous systems have been developed with more or less success over the last 20 years but have not been used thereafter (see reference in Voets *et al*., 2005). Indeed, these systems were too difficult to reproduce or utilize and the data generated on the growth and development of the symbiotic partners were limited (see Voets *et al*., 2005). It has however to be noticed that the tripartite system developed by Elmeskaoui *et al.* (1995) has allowed successful mycorrhization of strawberry and potato plantlets (see Louche-Tessandier *et al*., 1999; Hernández-Sebastià *et al*., 1999; 2000). However, in this system (1) the plants were grown in mixotrophic conditions and (2) mycorrhizal excised or transformed roots were present in the system. Therefore, Voets *et al*., (2005) and Dupré de Boulois *et al.,* (2006a) proposed two new *in vitro* culture systems in which AM fungi and an autotrophic plant were grown in association.

At present, the *in vitro* culture systems developed by Voets *et al*., (2005) and Dupré de Boulois *et al.,* (2006a) have been successfully used with three strains of *G. intraradices* (MUCL 41833, 43194 and 43204), *Glomus* sp. (MUCL 43195) and *G. clarum* (MUCL 46238)) grown in association with *Medicago truncatula* and *Solanum tuberosum*. As these systems have been recently developed, the possibility to grow other AM fungal species belonging to other genera in association with other plant species is currently under investigation. The procedures described below therefore aim at presenting 'typical' associations that have been obtained using these *in vitro* culture systems. Specific adaptations to other hosts or AM fungi might therefore be necessary.

In the section below, we will present the procedures to obtain AM fungus-plant associations in the systems of Voets *et al*., (2005) and Dupré de Boulois *et al.,* (2006a) with seeds of *Medicago truncatula* as plant starting material. Note however that microcuttings from *in vitro* micropropagated plantlets (see Voets *et al*., 2005) can also be used and offer the advantage that all plantlets have the same genetic profile.

Surface-sterilization/Scarification of seeds

Various methods have been developed to sterilize/scarify seeds. They can involve, for instance, concentrated sulfuric acid, sodium/calcium hypochlorite, or mercuric chloride. The procedure

described here can greatly vary between plant seeds due to the thickness of the seed coat or sensibility of the seeds.

Here we will present the sterilization/scarification of *M. truncatula* seeds using sodium hypochlorite as described by Dupré de Boulois *et al*. (2006a).

1. Place seeds into a disposable sterile 50ml Falcon tube;
2. Add 25ml of concentrated sodium hypochlorite (8% active chlorine);
3. Shake the falcon tube for 10 minutes. The seeds will slightly swell and the outer hard seed coat will break;
4. Remove the sodium hypochlorite;
5. Add 25ml of sterile distilled water to the seeds and mix gently for 10 minutes;
6. Remove the water and repeat the rinsing step twice;
7. Place the surface-sterilized seeds in Petri plates (10-15 seeds/Petri plate) containing 40ml solidified ($3g.l^{-1}$ GelGro^{TM}) MSR or M medium lacking both sucrose and vitamins;
8. Place the Petri plates in a temperature-controlled incubator (20°C) in the dark;
9. The seeds will germinate within 3-5 days.

Note that surfactant such as Tween-20 or 80 can be added to sodium hypochlorite to reduce surface tension and allow better surface contact.

Stratification (cold treatment) can be performed to uniform germination and growth, but this procedure is not compulsory and not necessary with *M. truncatula* as all seeds germinate within a short period of time.

1. After sterilization/scarification, place the Petri plate at 4°C instead of 20°C in the dark.
2. After 14 days, place the Petri plates at 20°C in the dark.
3. The seeds will germinate within 2-3 days.

Medium

Neither sucrose nor vitamins need to be included in the culture medium as the plants are photosynthetically active and can thus produce photosynthates. The host plant can therefore grow in total autotrophy.

In the works of Voets *et al*., (2005) and Dupré de Boulois *et al.,* (2006a), MSR medium or an MSR-based medium (MSR medium with low K content to study Cs transport) have been used, but K. Fernandez (pers. comm.) has successfully used other media based either on the MSR or MS medium to associate *S. tuberosum* with *G. clarum* in the system developed by Voets *et al*. (2005).

In the systems developed by Voets *et al*., (2005) and Dupré de Boulois *et al.,* (2006a), new medium needs to be added in the RC during the course of the experiment to support plant and AM fungal growth, but also to restrict medium depletion due to plant transpiration. Therefore, the RC of the systems cannot be filled as described for bi-compartmented ROC systems (see section 1.1.). In the following paragraphs (sections 2.3. and 2.4.), detailed explanation will be given for the two types of system.

The Half-closed Arbuscular Mycorrhizal-Plant (HAM-P) in vitro culture system (Voets et al., 2005)

Originally, the Half-closed AM-P (HAM-P) *in vitro* culture system (Figure 2) consisted of one compartment, i.e. a 90mm diameter Petri plate, in which the roots of an autotrophic plant and an AM fungus were associated (Voets *et al*., 2005), while the shoot developed outside this compartment under open-air conditions. Using this system, Voets *et al*. (2005) obtained several thousand spores, an extensive ERM and abundant root colonization. The spores produced in this system were able to colonize new plantlets under the same conditions and therefore underlined the capacity of this autotrophic culture system to continuously culture AM fungi. The HAM-P *in vitro* culture system was thereafter adapted to perform transport studies by using a bi-compartmented Petri plate instead of a mono-compartmented Petri plate, as shown in Figure 2, with a root compartment (RC) and a hyphal compartment (HC).

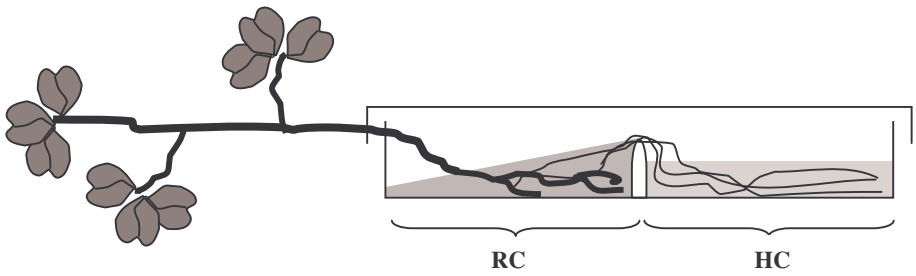
The set-up of this system is as follow:

1. A hole (± 2mm in diameter) is made using heated scalpel by melting (a) the edge of the lid to its top (2mm wide) and (b) the base of the Petri plate 2mm downwards (2mm wide). This hole is made in the middle of the RC, perpendicularly to the partition wall.
2. In the RC, 20ml of solid culture medium lacking both sucrose and vitamins is added at an angle of ± 4°, using a support for Petri plates, so that the medium reaches the top of the partition wall while on the opposite side of the RC the medium reaches a height of 2-3 mm (see Figure 2).
3. A germinated plantlet presenting vigorous growth and a strong primary root is transferred to the system. The roots are placed on the surface of the medium in the RC and the shoot is inserted in the hole previously made in the base of the Petri plate.
4. Approximately 100 spores are inoculated along the roots of the plantlet after their isolation as described in section 1.3.
5. The lid is placed on the Petri plate so that the cut edge of the lid is above the plantlet shoot. The hole is then carefully plastered with sterilized (121 °C for 15 min) silicon grease to avoid contamination.
6. The Petri plate is sealed with Parafilm™.
7. The Petri plate is covered with an opaque black plastic bag and incubated horizontally in a growth chamber set at 20°C with 70% relative humidity and 16/8h photoperiod. The

photosynthetic photon flux should ideally be comprised between 200 and 400 µmol.m^{-2}.s^{-1}.

8. Medium is regularly added (about 5ml once every one to three weeks) to the RC to insure plant and fungal growth, but also to insure that medium does not dry out. The medium is added at a temperature below 40°C in order to reduce the risk of damaging the roots and AM fungi.
9. When extensive spread of the ERM has been obtained, liquid or solid medium is added into the HC to ensure mycelium growth in this compartment (see section 1.1.).
10. Isotopic labelling and harvest are described in sections 3.3. and 3.5.

Figure 2 Schematic representation of the Half-closed Arbuscular Mycorrhizal-Plant (HAM-P) *in vitro* culture system viewed from the side. The system allows the spatial separation of a root compartment (RC) where roots and an AM fungus grow and a hyphal compartment (HC) in which only the extraradical hyphae are allowed to proliferate. The shoot develops in open-air conditions while the roots and AM fungus are maintained *in vitro* in the RC and HC of a bi-compartmented Petri plate (90 mm diam).



Remarks:

Sterility of the system: This system does not allow growth of both plant and AM fungi under strict *in vitro* conditions as the shoot

develops in open-air conditions. The shoot can therefore be subjected to various abiotic stresses and to possible contamination that could lead to the development of micro-organisms within the Petri plate (Dupré de Boulois *et al*., 2006a).

Depletion of the medium: Due to the transpiration of the host plant, rapid depletion of the medium in the RC occurs while the plant is actively growing. Regular addition of new medium is therefore necessary. However, plants may not deplete the medium at a similar rate and therefore is it difficult to add the same amount of medium to each HAM-P *in vitro* culture system. Furthermore, the rapid depletion of the medium in the RC is problematic as it may cause (1) difficulties for the ERM to cross the partition wall, (2) numerous extraradical hyphae to break after they had successfully crossed the partition wall and eventually (3) the necessity to add medium in the RC during the labeling period.

Labeling: As the shoot develops in open-air conditions, it can easily be labeled with gases (e.g. $^{13,14}CO_2$, $^{15}NO_2$) or any labeled molecules or elements that can be absorbed by the leaves or stem (e.g. sugars, fungicides, Cs, Zn). This represents a major advantage compared to the system developed by Dupré de Boulois *et al*. (2006a) described below.

The Arbuscular Mycorrhizal-Plant (AM-P) in vitro *culture system (Dupré de Boulois et al., 2006a)*

To overcome the limitations of the HAM-P *in vitro* culture system (i.e. sterility and medium depletion), Dupré de Boulois *et al*. (2006a) developed a complete Arbuscular Mycorrhizal-Plant (AM-P) *in vitro* culture system, in which both AM fungi and plant are maintained under strict *in vitro* conditions. The Arbuscular Mycorrhizal - Plant (AM-P) *in vitro* culture system (Figure 3) consists of three compartments, i.e. a shoot compartment (SC) where the shoot develops, a root compartment (RC) where roots and AM fungi grow and a hyphal compartment (HC) where only the ERM can proliferate. Using this system, Dupré de Boulois *et al.* (2006a) showed that AM fungi can take up, translocate and transfer Cs to *M. truncatula*. Transport of P was also studied and the results showed that 98.3% of the initial supply of ^{33}P in the HC was taken up by the ERM developing in the HC. Translocation to *M. truncatula* represented 91.7% of the ^{33}P taken up, and the distribution of ^{33}P in the mycorrhizal plantlets was 76.9% in the roots and 23.1% in the shoot. AM fungal growth parameters were high and similar to the ones obtained by Voets *et al*. (2005) using the HAM-P *in vitro* culture system. Spores produced in the AM-P *in vitro* culture system were viable and able to colonize new plantlets under the same conditions (unpublished data).

Preparation of the AM-P in vitro culture system

1. A hole (5 mm diameter) is made in the lid of the Petri plate using a cork-borer and the lower part of a Falcon tube (50 ml, Sarstedt Aktiengesellschaft and Co, Nümbrecht, Germany) is cut diagonally (hole of about 5 mm diameter) with a scalpel to obtain an angle of 65 ± 5° when placed on the lid of the Petri plate. The cork-borer and scalpel are heated with the flame of a Bunsen burner to facilitate the cuttings.
2. The Falcon tube and the Petri plate are then glued together using plastic thermo-fusible glue adapted to plastic gluing.
3. A hole (10 mm diameter) is made in the cap of the Falcon tube using a cork-borer, and a filter (18.6 mm diameter with inner efficient diameter for gas exchange of 10 mm and surface of 78.5 mm^2) is fixed on the cap. The filter used by Dupré de Boulois *et al*. (2006a) is an Adhesive Microfiltration Disc (AMD) in polypropylene laminated PTFE (Tissue Quick Plant Laboratory, Hampshire, UK) which prevents microbial contamination but allows gas exchange (Nominal Pore Size (NPS) of 0.25 µm). Note that other ventilation filters may be used such as the one made of cellulose or polyvinyl chloride.
4. The AM-P *in vitro* culture system is then sterilized at 25 kGy by gamma irradiation. Note that other sterilization procedures using for instance electron-beam (E-Beam)

sterilization can also be used, but it is not recommended to use ethylene oxide (EtO) sterilization as EtO can be absorbed by some plastics and must then be treated to eliminate any EtO before use. Note that autoclaving the AM-P *in vitro* culture system is not possible as thermofusible glue will melt.

Set up

In the work of Dupré de Boulois *et al*. (2006a), solid MSR^{Cs} medium (Declerck *et al*., 2003) lacking vitamins and sucrose was added in the Petri plate as described in Figure 1c, but two holes were made in the medium with a cork borer to allow addition of new medium during the course of the experiment to insure adequate mineral nutrition of plant and AM fungi. However, if this procedure was efficient (Dupré de Boulois *et al*., 2006a), it has to be noticed that due to water uptake by the mycorrhizal roots and transpiration, a slight medium depletion was observed (eq. to about 10ml of medium in ten weeks with *M. truncatula*) and probably restricted the capacity of fungal hyphae to cross the partition wall and thus develop in the HC. Therefore, we will present here another procedure.

1. In the RC, add 20ml of MSR medium lacking sucrose and vitamins.
2. A germinated plantlet presenting vigorous growth and a strong primary root presenting eventually lateral roots is

transferred to the system. The roots are placed on the surface of the medium in the RC and the shoot is inserted into the SC.

3. Approximately 100 spores are inoculated along the roots of the plantlet after their isolation as described in section 1.3.
4. The Petri plate is sealed with Parafilm or plastic wrap.
5. The Petri plate is then covered with an opaque black plastic bag and incubated horizontally in a growth chamber set at 20°C with 60% relative humidity and 16/8h photoperiod. The photosynthetic photon flux should be comprised between 200 and 400 $\mu mol.m^{-2}.s^{-1}$.
6. Medium is regularly added (about 5ml once every one to three weeks) to the RC to insure plant and fungal growth. The medium is added at a temperature below 40°C in order to reduce the risk of damaging the roots and AM fungi.
7. When an extensive spread of the ERM has been obtained, liquid or solid medium is added into the HC to ensure mycelium growth in this compartment (see section 1.1.).
8. Isotopic labelling and harvest are described in sections 3.3. and 3.5.

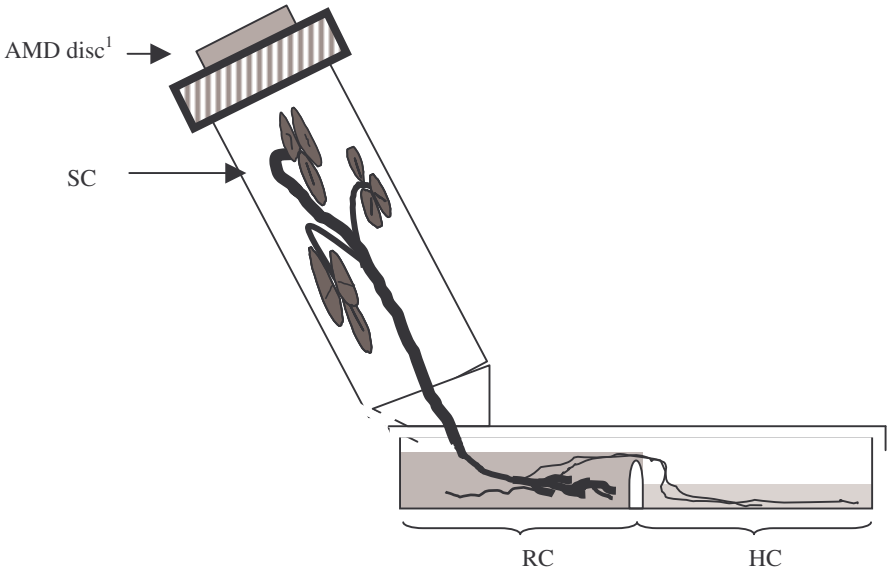
Remarks:

Depletion of the medium: Due to the transpiration of the host plant, depletion of the medium in the RC occurs while the plant is actively growing. However, this depletion is low as the transpiration of the plant is limited under these *in vitro* conditions. Regular addition of

new medium is nevertheless necessary to insure plant and AM fungal development. If other ventilation membranes or Petri plate sizes are used, it is necessary to assess medium depletion in order to have the RC filled at least as shown in Figure 1a when the ERM will extend enough to cross the partition wall between the RC and HC.

Plant transpiration: As the plants develop fully *in vitro* with only a membrane allowing gas exchange, it is possible that the relative humidity within the AM-P *in vitro* culture system is very high and limits plant transpiration. If this is advantageous as medium depletion in the RC is low, it may have significant impact on plant physiology such as plant photosynthesis, nutrient translocation from root to shoot or plant abscisic acid content.

Figure 3 Schematic representation of the Arbuscular Mycorrhizal - Plant (AM-P) AM-P *in vitro* culture system. The system allows the spatial separation of a shoot compartment (SC) where the stem and leaves develop, a root compartment (RC) where roots and hyphae grow and a hyphal compartment (HC) in which only the hyphae are allowed to proliferate (from Dupré de Boulois *et al*., 2006a). The RC and HC are constituted of a bi-compartmented Petri plate (90mm diam) and SC of a 50ml Falcon tube.



[1] Adhesive Microfiltration Disc (AMD) in polypropylene laminated PTFE avoiding contamination but allowing gas exchanges.

Labeling with isotopic tracers

Isotopic labeling is performed by using an isotopic tracer, which is an atom of an isotope used to observe the movement of certain elements in chemical, biological, or physical processes. The term tracer is applied commonly to any stable or radioactive isotope used to trace the course of non-radioactive elements or biological substances (e.g. proteins, DNA, glucides), but also radioactive elements such as

radionuclide contaminants. The observations may be conducted by the measurement of the relative abundance of isotopes if stable isotopes are used as tracers or of radioactivity in the case of radioactive tracers. Instruments used to detect stable isotopes include principally mass spectrographs and nuclear magnetic resonance (NMR) spectrometers. Instruments used to detect radiation may include liquid scintillation counters, gamma counters and (micro)-autoradiographs.

The isotopic tracer should be sterilized before addition into the Petri plate. Filter-sterilization using a 0.2µm filter is recommended.

Stable or radio-isotopic tracer?

The choice between stable and radioactive isotopic labeling depends on:

- The purpose of the experiment: if the goal is to demonstrate the presence of an element, both stable and radioactive isotopes can be used. However, if the goal is to study a metabolic process, a stable isotope should be used in combination with NMR spectrometry or mass spectrometry (MS).
- The availability of the stable and suitability of the radioactive isotope. Some elements have no stable isotope (i.e. technetium, tungsten and promethium and all elements with an atomic number greater than 82). Therefore, all tracers used to investigate these elements must be their

radioactive forms. Conversely, some elements have only radioactive isotopes with a very short half-life (i.e. inferior to a few days). Therefore, they cannot or with difficulties be used for transport studiess with a very short half-life (i.e. inferior to a few days). In this case the tracer must be a low-abundance stable isotope prepared in enriched form. Also, not all stable isotopes can be used with NMR spectrometry (i.e isotopes with a zero nuclear spin) or mass spectrometry (i.e. isobars). Note that isobars and isobar molecules can be distinguished using Accelerator Mass spectrometry (AMS) or Resonance-Ionization Mass Spectrometry (RIMS), but these instruments are only available in some highly specialized laboratories.

- The detection limit of the instruments: The choice between stable and radioactive isotopes can be determined by the so-called "dilution factor", which is a measure of the concentration of tracer required for detection. Generally, radioactive tracers may be detected in much lower quantities than stable tracers. For example, a pure ^{13}C tracer would be detectable after being diluted 100000 to 1 million times with natural ^{12}C. Radioactive ^{14}C, however, can be detected after being diluted 25 billion times with $^{12,13}C$.

For many studies, it is possible to choose between stable-isotope tracers and radioactive-isotope tracers. For example, it is possible to

choose between ^{13}C (stable) and ^{14}C (radioactive) or ^{31}P (stable) and $^{32,33}P$ (radioactive) isotopes.

Concentration of the isotopic tracer, radiotoxicity and chemotoxicity

The quantity of isotopic tracer used depends mainly on the physical half-life of the radioisotopic tracers and on the equipment available to detect them (i.e. counting efficiency, detection limit). Preliminary tests might therefore be necessary prior to experimentation.

Chronic internal exposure of some toxic elements at low levels is likely to cause responses distinct from those observed after acute exposure at high doses because of bioaccumulation. Biochemical mechanisms can indeed lead to a gradual accumulation of elements present at trace level in the external medium, inducing a highly localized deposit within tissues or cells. These highly localized accumulations may lead to either or both radiological (e.g. Cs) and chemical (e.g. As, Pb, Cd, Zn) toxicities, depending on whether stable or radioactive isotopes are used. Such exposure to a toxic element and its accumulation may give rise to particular biological responses of a cell group, capable of causing functional or structural abnormalities. Therefore, care should be taken to restrict the concentration of the isotopic element in the medium and its exposure with the AM fungi and host roots.

Adding the isotopic tracer

After the addition of medium in the HC, extraradical hyphae will develop rapidly and extend in the whole compartment. Generally, within 1 to 4 weeks the density of the ERM will be sufficient to expose it to the isotopic tracer. However, it is also possible to expose the ERM to the labeled medium contained in the HC as soon as it crosses the partition wall (see Table 3).

The isotopic tracer can be added to the HC either directly in/on the medium contained in this compartment depending whether liquid or solid medium is used, or after renewing it. Note that if the isotopic tracer is added on solid medium, its diffusion into the medium should be verified (using autoradiography for instance, Gray *et al*., 1995). Renewing liquid medium is done by gently removing the old medium using a pipette and adding new one. In the case of solid medium, a scalpel and forceps are used to cut and remove the medium. Thereafter, new medium is added in the HC. Solid medium should be cooled at a temperature below 40°C before its addition in order to reduce the risk of damaging the roots and AM fungi. The temperature of the liquid medium should be 25-27°C.

Renewing solid medium in the HC means that the AM fungal mycelium growing in the HC will be removed and might therefore stress the fungus. However, rapid development of AM fungal

mycelium is usually observed. This operation might be even useful to synchronize ERM growth in the HC and labeling.

It should be noticed that it is also possible to mix the isotopic tracer directly with the medium before addition in the HC. For some experiments, for instance on C transport, the isotopic tracer should be placed in the RC. As the medium cannot be removed, the isotopic tracer must then be added on the solidified medium.

To get a more accurate measure of the transport and metabolism processes, it can be useful to suppress from the medium the element (or a chemical analog) under study. For instance, Declerck *et al*. (2003) strongly reduced the concentration of K in the RC and had no K in the HC to study Cs transport. Identically, Jin *et al*. (2005) used medium lacking N but amended with ^{15}N labeled substrates to study N transport and metabolism in AM symbiosis. However, it should be taken into consideration that removing or modifying the concentration of one element from the culture medium will perturb the concentration of at least one other element and the balances between elements and may therefore impact the growth of the AM fungus and host.

Exposure time

There is no predefined time for contact between the ERM and the isotopic tracer. AM transport and biosynthetic pathways vary depending on the element or substances under study but also on the

fungal strain, age of the culture and ERM density in the HC. In table 3, a few representative examples are given for five isotopic tracers and with the four major techniques used to detect them.

Harvest

Before collecting the ERM from the HC containing liquid medium, the medium is removed using a manual or motorized pipettor, and the compartment is rinsed 2-3 times with water. The extraradical hyphae can then be harvested easily by shearing the extraradical hyphae along the partition wall using forceps and scalpel under the stereomicroscope.

If the HC contains solid medium, the harvest of the ERM is more difficult as separation can only be performed by dissolving the gel using citrate buffer (pH 6, 10mM: 1.8mM citric acid + 8.2mM sodium citrate) with the risk of cytoplasm leakage.

To harvest the roots and ERM in the RC, it is possible either to:

- Remove the roots from the solid medium using forceps and eventually rinse them with citrate buffer if medium remains attached to the roots. In this case, as the extraradical hyphae will break during the procedure, it is advised not to separate the ERM from medium;
- Place the medium containing the roots and ERM directly in citrate buffer. In this case, the ERM will still be attached to

the root, so hyphae will need to be sheared from the root using scalpel and forceps. Roots and ERM are then rinsed in water.

Table 3 Examples of use of the ROC and AM-P *in vitro* culture systems to track element transport and metabolism by AM fungi. Time between initiation of ERM development in the HC and labeling ($T_{between}$), exposure of the ERM and/or roots to an isotopic tracer are given as well as the *in vitro* model used and technique to detect the tracer. LSC: Liquid Scintillation counting; GC-MS: Gas Chromatography – Mass Spectrometry; NMR: Nuclear Mass Spectrometry.

Element or molecule studied	**($T_{between}$)**	**Exposure time**	***In vitro* model used**	**Technique used**	**Reference**
^{134}Cs ($^{134}CsNO_3$), ^{33}P ($^{}H_3{}^{33}PO_4$)	2 weeks	2 weeks	AM-P	Gamma counter, LSC	Dupré de Boulois *et al*., 2006a
^{134}Cs ($^{134}CsNO_3$)	2 weeks	2 weeks	ROC	Gamma counter	Dupré de Boulois *et al*., 2005a
^{15}N ($^{15}NO_3$, $^{15}NH_4{}^+$, arginine-guanido-^{15}N.HCl), ^{13}C ($^{13}C_{U6}$ arginine, $^{13}C_2$ acetate)	No	6 weeks	ROC	GC-MS	Govindarajulu *et al*., 2005
^{15}N ($^{15}NH_4Cl$, arginine-guanido-^{15}N.HCl, $^{13}C_{U6}$ arginine)	No	1, 3 and 6 weeks	ROC	GC-MS	Jin *et al*., 2005
^{13}C ($^{13}C_1$ glucose)	11-13 days	8 weeks	ROC	NMR & GC-MS	Pfeffer *et al*., 2004
$^{14}C_U$ glucose	2 weeks	1, 2, 4, 6 and 8 weeks	ROC	LSC	Pfeffer *et al*., 2004
^{137}Cs ($^{137}CsNO_3$)	1 weeks	4 weeks	ROC	Gamma counter	Declerck *et al*., 2003
^{33}P (H_3PO_4), ^{233}U ($^{233}UO_2(NO_3)_2$)	1 week	2 weeks	ROC	LSC	Rufyikiri *et al*., 2004
^{32}P ($H_3{}^{33}PO_4$, α-^{32}P-CTP)	3 weeks	7 hours to 20 days	ROC	Autoradiography & LSC	Nielsen *et al*., 2002
^{33}P ($H_3{}^{33}PO_4$)	4 weeks	6 to 48 hours	ROC	LSC	Maldonado-Mendoza *et al*., 2001
$^{32,33}P$ ($H_3{}^{33}PO_4$, 5' [α-^{32}P] AMP)	Not mentioned	3 days	ROC	LSC	Joner *et al*., 2000b

Conclusion

In the present chapter, we presented the bases to conduct transport studies using either the ROC or the newly developed HAM-P and AM-P *in vitro* culture systems. These systems represent powerful tools to investigate nutrient, trace element and C transport and metabolism by AM fungi. However, the utility of these systems should not be limited to transport studies. Indeed, as the HAM-P and AM-P *in vitro* culture systems have major advantages over the ROC, these systems will most probably become important models to investigate AM biology and AM fungus-plant interactions.

RESULTS

Chapter I

Extraradical mycelium of the arbuscular mycorrhizal fungus *Glomus lamellosum* can take up, accumulate and translocate radiocaesium under root-organ culture conditions

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Extraradical mycelium of the arbuscular mycorrhizal fungus *Glomus lamellosum* can take up, accumulate and translocate radiocaesium under root-organ culture conditions

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Abstract

Radiocaesium enters the food chain when plants absorb it from soil, in a process that is strongly dependent on soil properties and plant and microbial species. Among the microbial species, arbuscular mycorrhizal (AM) fungi are obligate symbionts that colonize the root cortex of many plants and develop an extraradical mycelial (ERM) network that ramifies in the soil. Despite the well-known involvement of this ERM network in mineral nutrition and uptake of some heavy metals, only limited data are available on its role in radiocaesium transport in plants. We used root-organ culture to demonstrate that the ERM of the AM fungus *Glomus lamellosum* can take up, possibly accumulate and unambiguously translocate radiocaesium from a ^{137}Cs-labelled synthetic root-free compartment to a root compartment

and within the roots. The accumulation of ^{137}Cs by hyphae in the root-free compartment may be explained by sequestration in the hyphae or by a bottleneck effect resulting from a limited number of hyphae crossing the partition between the two compartments. Uptake and translocation resulted from the incorporation of ^{137}Cs into the fungal hyphae, as no ^{137}Cs was detected in mycorrhizal roots treated with formaldehyde. The importance of the translocation process was indicated by the correlation between ^{137}Cs measured in the roots and the total hyphal length connecting the roots with the labelled compartment. ^{137}Cs may be translocated via a tubular vacuolar system or by cytoplasmic streaming *per se*.

Introduction

Since the early 1960s, intentional and accidental discharges from nuclear installations and from above-ground testing of thermonuclear weapons have resulted in substantial amounts of radiocaesium (^{134}Cs and ^{137}Cs) being released into the environment and incorporated into the food chain. ^{137}Cs poses considerable environmental problems because of its relatively long half-life, emission of β and γ radiation, rapid incorporation into biological systems (White and Broadley, 2000) and the similarity of its biogeochemical behaviour to potassium, a major plant nutrient (Delvaux *et al*., 2000).

The principal routes of radiocaesium entry into the food chain are atmospheric deposition on above-ground vegetation and the soil-to-plant pathway (Bell *et al*., 1988). Root uptake of radiocaesium is controlled by the selective sorption of trace Cs on specific binding sites (frayed edge sites borne by weathered mica) (Cremers *et al*., 1988; Delvaux *et al*., 2001), the concentration of potassium in the soil solution around plant roots (Smolders *et al*., 1996; 1997; Delvaux *et al*., 2001), the plant species (White and Broadley, 2000) and rhizosphere processes involving soil microorganisms (Gadd, 1996).

Soil microorganisms play a major role in virtually all biogeochemical cycles (Gadd, 1996). Arbuscular mycorrhizal (AM) fungi are intimately associated with plant roots and occupy an important position at the soil–root interface. Arbuscular mycorrhizal fungi colonize the root cortex inter- and intracellularly and develop an intricate extraradical mycelial (ERM) network in the soil surrounding most vascular plants (Smith and Read, 1997). This ERM network forms an active continuum from soil to plant, enabling the uptake of ions from the soil and their translocation into the host roots and transfer to other host tissues, these three steps being referred to as transport (Cooper and Tinker, 1978). Although AM fungi influence mineral nutrition (Smith and Read, 1997) and heavy metal sequestration/transport (Leyval *et al*., 1997; Kaldorf *et al*., 1999), little is known about their effects on radiocaesium uptake, translocation and transfer to plants. Increased accumulation of trace Cs was observed in leaf tissues of AM *Paspalum notatum* (McGraw *et al*., 1979),

Melilotus officinalis (Rogers and Williams, 1986) and *Festuca ovina* (Dighton and Terry, 1996), whereas a decrease was observed in AM *Trifolium repens* (Dighton and Terry, 1996) and *Agrostis tenuis* (Berreck and Haselwandter, 2001), and no effect was observed in AM *Sorghum sudanense* (Rogers and Williams, 1986). The difficulty in maintaining experimental systems devoid of organisms other than the two symbiotic partners has impeded the elucidation of the role of AM fungi in Cs transport. Recently, Rufyikiri *et al*. (2002) used root-organ culture (ROC) multicompartment growing systems to study the effects of the ERM on uranium transport. Based on the spatial separation of mycorrhizal roots and ERM ramifying in a neighbouring root-free compartment, this ROC system allowed an evaluation of the role of hyphae in the uptake and translocation of U and could, presumably, be used for the investigation of a wide range of radioelements, including Cs.

The objective of this study was to determine the effects of AM fungi on the uptake and accumulation of Cs under ROC conditions and to evaluate the role of ERM in Cs translocation into host roots.

Experimental procedures

Biological material

Agrobacterium rhizogenes-transformed carrot roots (*Daucus carota* L.) colonized with *Glomus lamellosum* Dalpé, Koske and Tews (MUCL 43195) and non-mycorrhizal carrot roots were purchased from Ginco (http://www.mbla.ucl.ac.be/ginco-bel). Both materials were provided in Petri plates (90 mm diameter) on modified Strullu–Romand (MSR) medium (Declerck *et al*., 1998) modified from Strullu and Romand (1986). Petri plates were incubated in an inverted position in the dark at 27°C. Root cultures were subcultured every 3 weeks, whereas the AM fungal cultures were incubated for 5 months. Several thousand spores were produced during this time.

Experimental set-up and growth conditions

Spores of *G. lamellosum* were isolated from the Petri plates by solubilization of the MSR medium (Doner and Bécard, 1991) and maintained in deionized sterile (121°C for 15 min) water. Spores (±100) were placed in the vicinity of transformed carrot roots (70 mm length) in Petri plates (90 mm diameter) with vertical wall separating the root and hyphal compartments (St Arnaud *et al*., 1995; Joner *et al*., 2000b). The root compartments (RC) were filled with 30 ml of an adapted MSR medium (termed MSR^{Cs}), containing in mg l^{-1} distilled water: $MgSO_4.7H_2O$, 739; $NaH_2PO_4.2H_2O$, 4.7; $Ca(NO_3)_2.4H_2O$, 359;

KCl, 11.46; NaFeEDTA, 8; $MnSO_4.4H_2O$, 2.45; $ZnSO_4.7H_2O$, 0.29; H_3BO_3, 1.86; $CuSO_4.5H_2O$, 0.24; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.035; $NaMoO_4.2H_2O$, 0.0024; thiamine, 1; pyridoxine, 0.9; nicotinic acid, 1; calcium panthotenate, 0.9; cyanocobalamine, 0.4; biotine, 0.9×10^{-3}; sucrose, 10 000 and solidified with agar (Aldrich agar fine powder; Sigma Aldrich Chemie) 5000 containing 180 mg kg^{-1} potassium. The total amount of K in this compartment was 0.21 mg and the concentration was 0.18 mM. The pH of the medium was adjusted to 5.5 before sterilization at 121°C for 15 min. The Petri plates were incubated in an inverted position at 27°C in the dark for 3 weeks. During this period, hyphae began to cross the vertical walls separating the compartments. The Petri plates were returned to a normal position for an additional week, and the hyphal compartments (HC) were filled with 27 ml of liquid MSR^{Cs} medium, lacking KCl and sucrose. Thus, no K was present in this second compartment. Roots that had crossed the vertical walls were carefully trimmed. After this week of incubation, the liquid MSR^{Cs} medium in the HC was removed and replaced with MSR^{Cs} medium (27 ml) lacking KCl and sucrose, but containing 390 Bq ml^{-1} ^{137}Cs. The ^{137}Cs was filter sterilized (Acrodisc® syringe filters; Pall) before it was added to the liquid medium. Petri plates were incubated for 4 weeks at 27°C in the dark. Identical Petri plates with 2% (v/v) formaldehyde were included as a control (formaldehyde control) to determine whether ^{137}Cs uptake and translocation by hyphae was an active process. After the mycelium had developed in the HC (4 weeks), formaldehyde was added and followed 3 days later by ^{137}Cs (390 Bq ml^{-1}). Two additional

treatments were also included: (i) Petri plates without roots and AM fungus (control); and (ii) Petri plates with roots in the RC (root control). Both were labelled with 390 Bq ml^{-1} ^{137}Cs in the HC. Ten replicates were considered for the experimental treatment, whereas four, six and three replicates where considered for the formaldehyde control, the control and the root control respectively.

Harvest and plant – AM fungus analysis

A non-destructive analysis of the replicates was first performed when ^{137}Cs was added, 4 weeks after mycorrhizal inoculation, and at the end of the experiment, i.e. 4 weeks after ^{137}Cs was added. Total ERM length and the number of spores in both RC and HC, and the total root length in the RC were assessed using a 10 mm grid of lines marked on the bottom of each Petri plate to form 10 mm squares. Vertical and horizontal lines were observed under a binocular microscope, and the presence of roots and hyphae was recorded at each point where they intersected a line. The total number of root or hyphae/gridline intersects was used to estimate the total length of roots (or hyphae) in a given area (Newman, 1966; see Giovannetti and Mosse, 1980) as $R = \pi NA/2H$, where N is the number of intersections, A the area within which the root or hyphae lies, H the total length of the straight lines, and R is the total length of roots or hyphae in the Petri plate. Spores were also counted in each cell formed by the gridlines (Declerck *et al*., 2001b) and totaled for each Petri plate.

A destructive analysis was performed at the end of the experiment. Hyphae crossing the barrier were cut. Hyphae in the HC were collected, rinsed in distilled water and subjected to ^{137}Cs counting (MINAXIγ Auto Gamma® 5000 Series, Gamma counter, Packard®). Liquid media in the HC were also sampled for ^{137}Cs counting. In the RC, the same process was followed. Roots were removed from solidified MSR^{Cs} medium with forceps and cleaned to free them from the remaining gel and external hyphae. Both the roots and the MSR^{Cs} medium including the gel and the ERM fungal structures were subjected to ^{137}Cs counting. After counting, the roots from the RC compartment were dried at 60°C for 48 h and weighed. Roots were stained for AM fungal root colonization measurements (Phillips and Hayman, 1970). Roots were cleared in 10% KOH at 60°C for 6 h, washed with distilled water and stained with 0.2% Trypan blue for 30 min at 60°C. The frequency of AM fungi colonization (%F) was estimated as the percentage of root segments (n = 50, 10 mm length) that were colonized by 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 estimated using the method outlined by Plenchette and Morel (1996).

Statistical analysis

Statistical analyses were performed with SAS. Data were subject to ANOVA, and significance between treatment means was ranked by

the Scheffé's multiple range test at $P < 0.05$. Independence between variables was assessed using a *t*-test for correlation coefficients with H0: $\rho = 0$, and H1: $\rho \neq 0$.

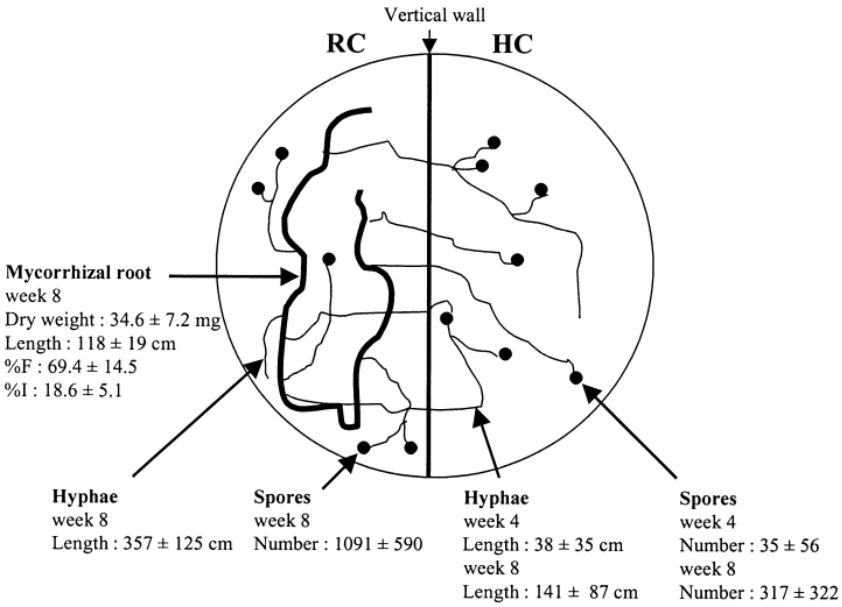
Results

Distribution of fungal biomass in the root compartment (RC) and hyphal compartment (HC) and intra- and extraradical development of AM fungal structures.

Percentage root length colonized by the AM fungus was high in the experimental treatment (Fig. 1), with typical hyphae and arbuscules present. Vesicles were also observed, but were rarely abundant. Fungal growth variables, estimated by hyphal length and spore number, together with plant growth variables, estimated by root length and dry weight, in RC and HC, is shown in Fig. 1. The root length (≈ 70 mm at the start of the experiment) increased 17-fold during the experiment and was characterized by long elongation zones +and a pyramidal pattern of lateral roots, demonstrating the vigour of root growth in the MSR^{Cs} medium. In both RC (solid) and HC (liquid) compartments, the hyphae spread into the complete volume of the Petri plate. Branched absorbing structures (BAS) (as described by Bago *et al*., 1998) were regularly observed along runner and lower order hyphae. Spores were observed in both compartments. Their maximum size ranged from 80 to 100 µm and did not differ between compartments. Hyphal length and spore number measured in the HC

increased from week 4 (^{137}Cs inoculation) to week 8 (harvest) by 3.7-fold and ninefold respectively. In the formaldehyde control treatment, before formaldehyde incorporation, i.e. at week 4, hyphae length and spore number in the HC reached 54.5 ± 19.9 cm and 18 ± 26 cm, respectively, whereas in the RC, hyphae length and spore number were 163.2 ± 27.7 cm and 136 ± 39 cm respectively. These values did not differ significantly from those measured at week 4 in the experimental treatment. After formaldehyde incorporation into the HC, no additional root and fungal growth was observed in the RC or in the HC. The frequency %F and intensity %I of root colonization measured at week 8 in this formaldehyde control treatment were 34.5 ± 12.3% and 12.1 ± 1.2% respectively. These values were significantly lower than the values in the experimental treatment.

Fig. 1. Growth variables of *Glomus lamellosum* associated with transformed carrot roots in bicompartmented Petri plates. RC (root compartment) data represent the growth of mycorrhizal roots and extraradical hyphae and spore production; HC (hyphal compartment) data represent extraradical hyphae and spore production. Root growth was evaluated by dry weight and length, whereas *Glomus lamellosum* development was evaluated by the frequency (%F) and intensity (%I) of root colonization, extraradical hyphae length and spore number produced outside the root. Variables in the HC were evaluated at week 4 (date of ^{137}Cs labelling of HC) and week 8 (end of experiment), whereas variables in the RC were evaluated only at week 8. Data are means of 10 replicates with standard deviation.

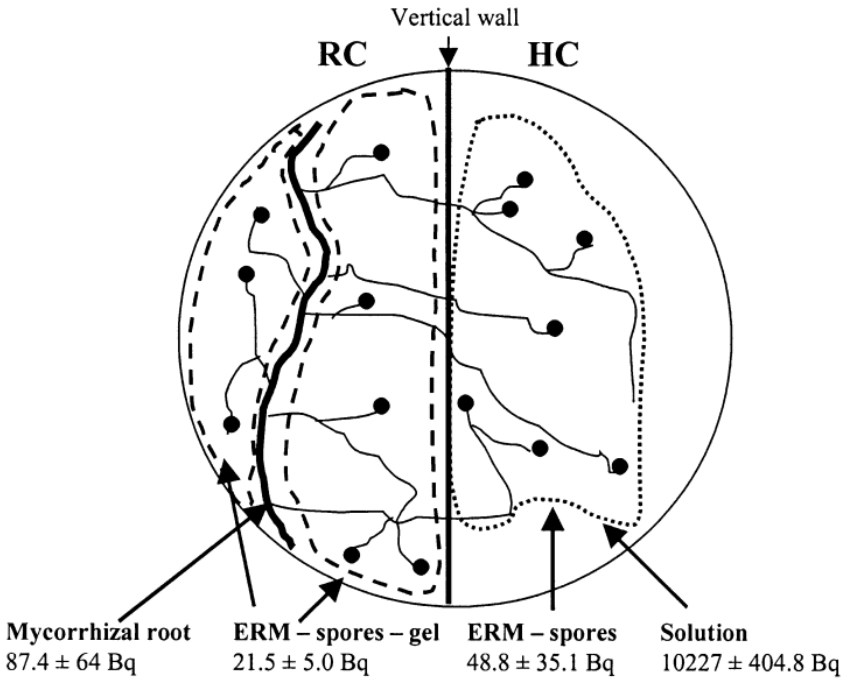


^{137}Cs uptake, accumulation and translocation by hyphae

Hyphal ^{137}Cs uptake and accumulation within the HC and translocation from HC to RC via the ERM was evident (Fig. 2) in the experimental treatment. Total ^{137}Cs content in the roots was 0.85% of the initial ^{137}Cs in the HC. This activity was 30-fold larger than in the root control (2.9 ± 1.7 Bq in roots). No ^{137}Cs content was measured in the roots of the formaldehyde control. Similarly, the total ^{137}Cs content measured in the medium of the RC, i.e. ERM, spores and gel,

of the experimental treatment was significantly larger (≈ 60-fold) than the root control (0.36 ± 0.15 Bq) and the control (0.35 ± 0.12 Bq), whereas no ^{137}Cs was found in the medium of the formaldehyde control. The ^{137}Cs content in the RC medium accounted for 0.21% of the initial ^{137}Cs in the HC. The ^{137}Cs content measured in the hyphae and spores of the AM fungus in the HC accounted for 0.47% of the isotope initially added to the HC, whereas in the formaldehyde control, this ^{137}Cs content accounted for only 0.004% (0.72 Bq in hyphae). Expressed in Bq cm^{-1} hyphae, the ^{137}Cs content in the experimental treatment, measured in the HC (0.34 Bq cm^{-1} hyphae), was significantly larger than the ^{137}Cs content measured in the RC (0.066 Bq cm^{-1} hyphae).

Fig. 2. Distribution of ^{137}Cs (expressed in Bq) in the extraradical structures of *Glomus lamellosum* and in mycorrhizal transformed carrot roots, grown in bicompartmented Petri plates. RC (root compartment) data are presented for ^{137}Cs accumulation in the mycorrhizal root and in the ERM, spores and gel; HC (hyphal compartment) data are presented for ^{137}Cs accumulation in the ERM and spores and in the solution. The ^{137}Cs content was determined 4 weeks after ^{137}Cs addition to the HC solution. Data are means of 10 replicates with standard deviation.



Correlation of structural parameters of the AM fungus and ^{137}Cs translocation

The total ^{137}Cs content measured in roots was positively correlated with the ^{137}Cs content in the RC medium, i.e. ERM, spores and gel ($r = 0.89$ with $P = 0.005$), and with the total hyphal length ($r = 0.83$ with $P = 0.02$) and total spore number ($r = 0.91$ with

$P = 0.003$) counted in the RC and HC. Similarly, the ^{137}Cs content expressed mg^{-1} root dry weight was positively correlated with the ^{137}Cs content in the RC medium, i.e. ERM, spores, gel ($r = 0.88$ with $P = 0.007$) and with the total hyphal length ($r = 0.84$ with $P = 0.03$) and total spore number ($r = 0.90$ with $P = 0.004$) counted in the RC and HC. No correlation was observed between the ^{137}Cs content measured in roots and AM fungal root colonization, with %F ($r = 0.55$ with $P = 0.401$) or %I ($r = 0.56$ with $P = 0.369$). The total ^{137}Cs content measured in the hyphae growing into the HC was positively correlated with the hyphal length measured in this compartment ($r = 0.92$ with $P = 0.002$).

Discussion

Previous studies of AM fungal transport of radiocaesium (McGraw *et al*., 1979; Rogers and Williams, 1986; Dighton and Terry, 1996; Berreck and Haselwandter, 2001) were conducted in pots, on plants in which the root system (mycorrhizal or not) was in close physical contact with the radionuclide, and developed in the presence of microorganisms other than the inoculated AM fungi. These protocols preclude the determination of whether the ERM of AM fungi can effectively translocate radiocaesium to roots.

We found that the ERM of an AM fungi can take up, possibly accumulate and unambiguously translocate radiocaesium from a

labelled root-free compartment to a root compartment, under ROC conditions, i.e. in the absence of any microorganisms other than the two partners of the symbiosis. This ability is consistent with the presence of ^{137}Cs in the RC, which was initially ^{137}Cs free, both in the MSR^{Cs} medium, i.e. ERM, spores and gel, and in the mycorrhizal roots.

The accumulation of ^{137}Cs by hyphae in the HC may be explained by internal sequestration by the extraradical hyphae as hypothesized by Berreck and Haselwandter (2001). Adsorption on negatively charged constituents of fungal tissues is another possible mechanism suggested for some metals (Joner *et al*., 2000a), and this hypothesis should be tested for ^{137}Cs. However, as the mycelium within the HC and RC was coenocytically interconnected, the difference in ^{137}Cs content in hyphae of the HC versus hyphae of the RC could result from a 'bottleneck' effect. Hyphae (data not collated) crossing the partition between the two compartments ramified extensively within the HC. Thus, translocation of ^{137}Cs to the RC was restricted by a limited number of hyphae relative to those in the HC, resulting in a possible bottleneck effect and accumulation in the hyphae in the HC. This hypothesis is supported by the recent findings of Nielsen *et al*. (2002) who demonstrated, in an identical ROC system, that the amount of P translocated from HC to RC was positively correlated with the number of hyphae crossing the partition.

The mechanisms governing the uptake and translocation of ^{137}Cs by and throughout extraradical mycelium remain speculative. However, translocation at least resulted from the incorporation of ^{137}Cs into the fungal hyphae, as no ^{137}Cs was detected in the mycorrhizal roots and MSR^{Cs} medium of the cultures treated with formaldehyde. Similar observations were obtained with ^{32}P (Joner *et al*., 2000b) and ^{233}U (Rufyikiri *et al*., 2002). Furthermore, translocation of ^{137}Cs to roots was strongly correlated with the total hyphal length connecting roots with the labelled compartment, supporting the ^{137}Cs translocation capacity of ERM. Mechanisms similar to K uptake and translocation may be used for Cs uptake and translocation, as these alkali metals have a high degree of chemical similarity (White and Broadley, 2000). Translocation in fast-moving vacuoles as a possible route for element traffic and cytoplasmic streaming *per se* may channel ^{137}Cs translocation. The work conducted by Orlovich and Ashford (1993) with *Pisolithus tinctorius*, an ectomycorrhizal fungus, provides strong evidence for the association of vacuolar P with K in extraradical hyphae. These authors also suggested translocation of these elements through the hyphae via a motile and tubularly interconnected vacuole, a movement independent of cytoplasmic streaming. Long-distance movement of K that was dependent on the simultaneous translocation of P has also been observed with *Paxillus involutus* (Jentschke *et al*., 2001). Recently, Uetake *et al*. (2002) reported the existence of a similar tubular vacuolar system in *Gigaspora margarita*, an AM fungus. These tubular vacuoles were observed in germ tubes and in extra- and

intraradical hyphae, but neither the motility of these structures nor their importance in intracellular translocation could be assessed. Another study (Nielsen *et al*., 2002), conducted on the same ROC system that we analysed, suggested that P may be translocated in either vacuoles or vesicles. Thus, a similar mechanism for transport of ^{137}Cs cannot be excluded.

Movement via cytoplasmic streaming is also a possible route for ^{137}Cs translocation. We observed bidirectional streaming microscopically. The fresh weight of the mycelium in our experiment was probably lower than the fresh weight of the root. Thus, the ^{137}Cs content mg^{-1} fresh weight mycelium would be higher than the ^{137}Cs content mg^{-1} fresh weight root, allowing a concentration gradient from hyphae in the HC via RC to the root.

Both cytoplasmic streaming and the presence of tubular vacuoles occur in extraradical hyphae of *Gi. margarita* (Uetake *et al*., 2002). Thus, both mechanisms could be involved in the translocation of ^{137}Cs.

Translocation from HC to RC accounted for < 1% of the isotope added to the HC. This low percentage could result from the intrinsic properties of hyphae with respect to translocation of ^{137}Cs. The chemical similarity of Cs to K and the observations that K is found at lower concentrations in the tissues of mycorrhizal plants than in non-mycorrhizal plants (George *et al*., 1992; Berreck and Haselwandter,

2001) suggest that a similar process is used for Cs (Berreck and Haselwandter, 2001).

Our experimental approach is not sufficiently precise to determine whether the ^{137}Cs translocated within the root was sequestered within the fungal intraradical structures, i.e. hyphae, arbuscules and vesicles, or if ^{137}Cs was transferred to the root cells. Restricted transfer of elements from fungal to root cells as a result of fungal sequestration was hypothesized for some metals (Kaldorf *et al*., 1999) and has been suggested for Cs (Berreck and Haselwandter, 2001). Microbeam analysis to map the distribution of heavy metals within mycorrhizal roots (Kaldorf *et al*., 1999) could be used to localize Cs within mycorrhizal roots.

In conclusion, our results suggest that the ERM of an AM fungus can take up, possibly accumulate and unambiguously translocate ^{137}Cs to the roots. More detailed observations of the localization of the radioisotope within the root is needed to resolve the question of transfer of ^{137}Cs from fungal structures within roots to root cells, and to assess the sink strength of this excised root culture system. In its present form, however, the system opens the possibility of investigations on the mechanisms involved in uptake, accumulation and translocation of Cs. Finally, as suggested recently for pesticides (Fortin *et al*., 2002) and for P (Nielsen *et al*., 2002), this system could become a standardized bioassay to assess the impact of AM fungi on other radionuclides and toxic elements in general.

Chapter II

Effects of arbuscular mycorrhizal fungi on the root uptake and translocation of radiocaesium

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Environmental Pollution 134(3): 515-524

Effects of arbuscular mycorrhizal fungi on the root uptake and translocation of radiocaesium

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Abstract

Because mycorrhizal fungi are intimately associated with plant roots, their importance in radionuclide (RN) recycling and subsequent dispersion into the biosphere has received an increasing interest. Recently, the capacity of arbuscular mycorrhizal fungi to take up and translocate radiocaesium to their host was demonstrated. However, the relative contribution of these processes in comparison to the ones of roots remains unknown. Here, the respective contributions of the hyphae of a *Glomus* species and the transformed carrot (*Daucus carota* L.) roots on radiocaesium uptake and translocation were compared and quantified.

We observed that radiocaesium uptake by hyphae was significantly lower as compared to that of the roots, while the opposite

was noted for radiocaesium translocation/uptake ratio. We also observed that the intraradical fungal structures might induce a local accumulation of radiocaesium and concurrently reduce its translocation within mycorrhizal roots. We believe that intraradical fungal structures might induce the down-regulation of radiocaesium channels involved in the transport processes of radiocaesium towards the xylem.

Introduction

After intentional or accidental discharges of radiocaesium (^{134}Cs and ^{137}Cs) in the environment, from nuclear installations and above-ground testing of thermo-nuclear weapons, the soil became its world major reservoir (Strebl *et al*., 1999 and McGee *et al*., 2000). Due to the low mobility of radiocaesium in soils (Bunzl *et al*., 1992 and Rosén *et al*., 1999), this radio-pollutant accumulates in the topsoil (Rafferty *et al*., 2000), which is intensively explored by roots for nutrients and water uptake. The chemical similarity of radiocaesium and potassium constitutes a major threat to radiocaesium contamination of the above-ground vegetation (Korobova *et al*., 1998) as their root uptake mechanisms appear to be closely related (White and Broadley, 2000). As a consequence, one of the principal routes for radiocaesium entry into the food chain is via the soil-to-plant pathway (Coughtrey and Thorne, 1983).

Soil binding properties of radiocaesium and the concentration of K in the soil solution have a major influence on radiocaesium root uptake (see review by Delvaux *et al*., 2001). At concentration of K below 1 mM, root uptake of radiocaesium significantly increases (Cline and Hungate, 1960 and Smolders *et al*., 1996) and is directly governed by soil binding properties (Delvaux *et al*., 2000). It was also observed by Zhu *et al*. (2000) that K starvation results in a stimulation of radiocaesium uptake which could be explained by an increased expression of high-affinity K transporters under such conditions (Santa-María *et al*., 1997 and Kim *et al*., 1998) as suggested by White and Broadley (2000). Recently, White *et al*. (2004) demonstrated that significant radiocaesium influx could be mediated through these transporters and confirmed their importance in radiocaesium uptake at low K concentration in the soil solution. Variations in radiocaesium soil-to-plant transfer have been reported among and within plant species (Coughtrey and Thorne, 1983, Desmet *et al*., 1990, Frissel *et al*., 2002 and Payne *et al*., 2004) supporting the influence of plant genotype in radiocaesium uptake (Buysse *et al*., 1996). Furthermore, Broadley *et al*. (1999) demonstrated that variations of radiocaesium accumulation in shoot could be identified at different taxonomic levels. Rhizospheric processes involving soil microorganisms also influence root uptake of radiocaesium (Gadd, 1996). In this respect, the obligate arbuscular mycorrhizal (AM) fungal symbionts are expected to play a key role (Entry *et al*., 1996 and Entry *et al*., 1999). AM fungi are intimately associated with plant roots and thus occupy an essential position at the soil/root interface. After colonizing the root

cortex inter- and intra-cellularly, the AM fungi develop an important biomass in the form of a network of extraradical hyphae. The *continuum* between extraradical hyphal network and intraradical fungal structures results in an active symbiotic bi-directional transport of carbohydrates and nutrients such as P from plant to AM fungi and from AM fungi to plant, respectively (Smith and Read, 1997). Recently, Declerck *et al*. (2003) demonstrated that the extraradical hyphal network of an AM fungus can take up and translocate radiocaesium from a radiocaesium labelled root-free compartment to a root compartment, using a monoxenic multi-compartment growing system (Fortin *et al*., 2002). This system was reported suitable for nutrient (Hawkins *et al*., 2000, Joner *et al*., 2000b and Nielsen *et al*., 2002) and radionuclide (Rufyikiri *et al*., 2002 and Rufyikiri *et al*., 2003) transport studies as well as for the identification of transport mechanisms (Fortin *et al*., 2002). The results of Declerck *et al*. (2003) along with pot culture experiments (Mc Graw *et al*., 1979, Rogers and Williams, 1986, Dighton and Terry, 1996, Strandberg and Johansson, 1998 and Entry *et al*., 1999) indicated that the AM fungi participate in radiocaesium transport from soil to plant. However, no study has yet compared and quantified the respective contribution of extraradical hyphal network, roots, and both in combination, to radiocaesium uptake and translocation. The quantification of the relative importance of the AM fungi and host root to radiocaesium uptake and translocation may be useful to identify their respective role in radiocaesium accumulation into plants and possibly to delineate

remedial strategies oriented towards phytostabilization or phytoextraction.

Materials and methods

Biological material

A *Glomus* sp. (formerly characterized as *Glomus lamellosum* Dalpé, Koske and Tews (MUCL) 43195) grown in association with Ri T-DNA transformed carrot (*Daucus carota* L.) roots and non-mycorrhizal Ri T-DNA transformed carrot roots were used. Both biological materials were purchased from GINCO (http://www.mbla.ucl.ac.be/ginco-bel) and delivered in Petri plates (90 mm diameter) on the modified Strullu–Romand (MSR) medium (Declerck *et al*., 1998 modified from Strullu and Romand, 1986), but solidified with 3 g l^{-1} GelGro™ (ICN, Biomedicals, Inc., Irvine, CA, USA). Petri plates were incubated in an inverted position in the dark at 27 °C. Roots were sub-cultured every three weeks, while AM fungal monoxenic cultures were incubated for five months. Several thousand spores were produced during this period.

Preparation of starter cultures

Four weeks before the beginning of the experiment, Ri T-DNA transformed carrot roots (70 mm long) were placed on the surface of

Petri plates (90 mm diameter) containing 40 ml MSR medium. Half of them were inoculated with the monoxenically produced spores (±100) of *Glomus* sp., following the method described by Declerck *et al*. (2003). The Petri plates, containing each one transformed carrot root with/without spores of *Glomus* sp., were subsequently incubated in an inverted position in the dark at 27 °C to produce mycorrhizal/non-mycorrhizal young active-growing roots.

Experimental design

Two-compartment Petri plates (90 mm diameter) were filled with MSR^{Cs} medium (Declerck *et al*., 2003) so that the medium extended 2 mm above the partition wall (Rufyikiri *et al*., 2003). This medium contained in mg L^{-1} distilled water: $MgSO_4 \cdot 7H_2O$, 739; $NaH_2PO_4 \cdot 2H_2O$, 4.7; $Ca(NO_3)_2 \cdot 4H_2O$, 359; KCl, 11.46; NaFeEDTA, 8; $MnSO_4 \cdot 4H_2O$, 2.45; $ZnSO_4 \cdot 7H_2O$, 0.29; H_3BO_3, 1.86; $CuSO_4 \cdot 5H_2O$, 0.24; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.035; $NaMoO_4 \cdot 2H_2O$, 0.0024; thiamine, 1; pyridoxine, 0.9; nicotinic acid, 1; calcium panthotenate, 0.9; cyanocobalamine, 0.4; biotine, 0.9×10^{-3}; sucrose, 10,000 and solidified with agar (Aldrich agar fine powder, Sigma Aldrich Chemie, GmbH, Steinheim, Germany) 5000 containing 180 mg kg^{-1} of potassium. After solidification, the medium in one compartment was cut along the partition wall with a scalpel and further removed by using a spatula. The volume of MSR^{Cs} medium in the remaining compartment was 30 ml. The total amount of K in this compartment was 0.21 mg and its concentration was 0.18 mM.

One-month old mycorrhizal and non-mycorrhizal root apexes (30 mm long), removed with scalpel and forceps from the starter cultures, were transferred into the compartments containing the MSR^{Cs} medium. This compartment was referred to as the culture compartment (CC). The Petri plates were then incubated in an inverted position in the dark at 27 °C. Hyphae and/or roots that crossed the partition wall separating the two compartments were trimmed whenever necessary.

After four weeks, the Petri plates were turned back to a normal position and 25 ml liquid MSR^{Cs} medium, lacking sucrose, vitamins and KCl (no K was present in this compartment to prevent any possible competition between K and Cs for uptake), was introduced in the second compartment. This compartment was referred to as the experimental compartment (EC). The Petri plates were then separated into three groups according to the treatment applied to the EC: (i) a hyphal compartment (HC) treatment where only extraradical hyphae were allowed to grow, (ii) a root hyphal compartment (RHC) treatment where both mycorrhizal roots and hyphae were allowed to grow, and (iii) a root compartment (RC) treatment where non-mycorrhizal roots were allowed to grow. For the HC treatment, roots that crossed the partition wall were trimmed to maintain the HC void of root. The Petri plates were incubated at 27 °C for two weeks before labelling.

Radiocaesium labelling and control treatments

Six weeks after the beginning of the experiment, the liquid medium contained in the ECs was removed with a sterile syringe and replaced with 5 ml of a fresh liquid MSR^{Cs} medium also lacking sucrose, vitamins and KCl. In half of the Petri plates of each treatment, formaldehyde (2% v/v) was introduced in the ECs two days before radiocaesium labelling in order to block metabolic activity of hyphae and/or roots. The formaldehyde treatment (Formaldehyde control) was included to determine whether the translocation of radiocaesium from the ECs to the CC was mediated by active processes involving the hyphae and/or the roots. Filter-sterilized (Acrodisc® Syringe Filters, PALL Corporation, Ann Arbor, MI, USA) radiocaesium (^{134}Cs) was then added to the liquid medium of all the Petri plates. The concentration of ^{134}Cs in the medium was estimated to 2062 ± 24 Bq ml^{-1} by performing 6 random tests during the labelling. Two additional control treatments were included in the experimental design: Petri plates without roots and AM fungi (Control) and Petri plates with only root in the CC (Root-control). These controls were not labelled with radiocaesium but half of them received formaldehyde as described above. These treatments aimed to determine possible γ background activity. All Petri plates were incubated for two weeks in the dark at 27 °C.

Harvest and plant – AM fungal analyses

At the end of the experiment (i.e. 8 weeks after the beginning of the experiment), the total extraradical hyphal length, root length and number of spores in the CC and ECs of all the Petri plates were estimated. The extraradical hyphae and root length were determined by using a 1 cm grid of lines marked on the bottom of each Petri plate to form 1 cm squares. Vertical and horizontal lines were observed under dissecting microscope and the presence of roots and hyphae recorded at each point where they intersected a line. The extraradical hyphae and root length were then calculated using the formula of Newman (1966) (see Declerck *et al*., 2003). Spores were counted as described by Declerck *et al*. (2001b). The number of active hyphae (i.e. observed under microscope (×40) by a bi-directional flux of cytoplasm/protoplasm) and roots crossing the partition wall between the two compartments were also counted under dissecting microscope.

The liquid solutions in the ECs were sampled for ^{134}Cs activity measurements. These compartments were rinsed twice with 10 ml distilled water, before hyphae and roots developing into them were collected individually. In the CC, roots were removed from the solidified MSR^{Cs} medium and cleaned-free from the remaining gel and external hyphae. The solidified MSR^{Cs} media containing the extraradical hyphae were then collected. Root fresh weights were measured. All samples were subjected to ^{134}Cs counting using a Germanium–Lithium detector operated with an AccuSpec/A®

multichannel analyzer and the Genie-2000 Spectroscopy System® software (Canberra Industries, Meriden, CT, USA). Radiocaesium (^{134}Cs) activity was measured at 605 keV with an optimal counting efficiency of 3.3%.

After counting, the mycorrhizal roots were placed in 10% KOH for clearing and stained with 0.2% Trypan blue (Phillips and Hayman, 1970) for measurement of root AM fungal colonization. Roots were cut into 10 mm segments and 50 randomly selected segments were examined under microscope for the evaluation of frequency (*%F*) and intensity (*%I*) of AM fungal colonization (Plenchette and Morel, 1996).

Statistical analysis

Statistical analysis of data was performed with the statistical software Statistica® for Windows (StatSoft, 2001). Data normally distributed and having homogeneous variances were subjected to analysis of variance (ANOVA). If a statistical difference ($P \leq 0.05$) was observed, the Tukey HSD (Honest Significant Difference) test was used to identify the significant differences ($P \leq 0.05$ and $P \leq 0.01$) among the individual groups. Independence between variables ($P \leq 0.05$) was assessed by using a *t*-test for correlation coefficients with H0: $\rho = 0$ and H1: $\rho \neq 0$. Values expressed in percentage were arcsin transformed before statistical analyses were performed.

Results

Roots and AM fungal growth variables

Root growth variables, estimated by root length and root fresh weight, together with fungal growth variables, estimated by frequency (*%F*) and intensity (*%I*) of fungal colonization, hyphal length and number of spores, in the CC and EC, without or with formaldehyde, are presented in Table 1. In the CC of the treatments without formaldehyde, root growth was characterized by long elongation zones and a pyramidal pattern of lateral roots. Root length (30 mm at the start of the experiment) averaged over HC, RHC and RC (i.e. 149 ± 9.6 cm) increased nearly 50-fold during the experiment, demonstrating the vigor of root growth on the MSR^{Cs} medium. Some roots, in the range of 1–4 and 1–3 for the RHC and RC treatments, respectively, crossed the partition between the two compartments, and developed in the ECs. Root length in the ECs averaged 21 cm ± 2.2 and 21 ± 5.6 for RHC and RC, respectively.

In both the CC and EC compartments, the hyphae spread into the complete volume of the Petri plates. Branched absorbing structures (BAS) (as described by Bago *et al*., 1998) and anastomoses (as described by Giovannetti *et al*., 1999) were observed along runner and lower order hyphae in both compartments. Hyphal length and spore production were high in the CC of the HC and RHC treatments, but did not differ significantly between these two treatments. The number

of active hyphae crossing the partition wall was high and ranged from 20 to 74 and 20 to 48 at harvest, in the HC and RHC treatments, respectively, but did not differ significantly between these two treatments. Hyphal length and spore production in the ECs, averaged over the treatments HC and RHC, were 944 ± 139 cm and 240 ± 50 individuals, respectively.

The *%F* and *%I* of root colonization in the CC were significantly higher in the HC treatment (81 ± 4.4% and 21 ± 2.1%, respectively) as compared to the RHC treatment (57 ± 7.5% and 12 ± 0.62%). In the EC of the RHC treatment, the *%F* and *%I* were 72 ± 11% and 24 ± 3.8%, respectively. Typical hyphae, arbuscules and vesicles were observed, although the latter were seldom abundant.

In the formaldehyde control treatment, hyphae and roots were killed after addition of formaldehyde in the ECs. Neither root, hyphal growth nor spore production was observed in the CC and ECs. Moreover, observations of the hyphae crossing the partition wall revealed that cytoplasmic flux had stopped totally within minutes after formaldehyde addition.

Table 1 Arbuscular mycorrhizal fungal growth variables, estimated by the frequency (%F) and intensity of fungal colonization (%I), hyphal length and number of spores, and root growth variables, estimated by root length and root fresh weight, in the culture compartment (CC) and in the experimental compartments (ECs) for the hyphal compartment (HC) treatment, the mycorrhizal root and hyphal compartment (RHC) treatment and the root compartment (RC) treatment, without or with formaldehyde added to the solution in the ECs.

	Without formaldehyde			With formaldehyde		
	HC N[a] = 7	**RHC** N = 6	**RC** N = 6	**HC** N = 8	**RHC** N = 7	**RC** N = 6
CC						
%F	81a ± 4.4[b]	57bc ± 7.5	-	52c ± 3.1	76ab ± 3.4	-
%I	21a ± 2.1	12bc ± 0.62	-	11c ± 0.26	15b ± 0.87	-
Hyphal length (cm)	1588ab ± 110	1682a ± 257	-	1076b ± 71	1163ab ± 71	-
Number of spores	1851a ± 133	1877a ± 363	-	1105a ± 138	1239a ± 104	-
Root length (cm)	152a ± 16	158a ± 13	129a ± 21	113a ± 19	117a ± 7	104a ± 19
Root fresh weight (mg)	445a ± 42	406a ± 36	380a ± 47	289a ± 47	340a ± 32	350a ± 62
Number of hyphae crossing the wall	39a ± 8	34a ± 4 -		35a ± 4.3	21a ± 3.3	-
Number of roots crossing the wall	-	2.5a ± 0.50	2.2a ± 0.31	-	2.4a ± 0.20	2.3a ± 0.33
EC						
%F	-	72a ± 11	-	-	76a ± 7.2	-
%I	-	24a ± 3.8	-	-	19a ± 2.3	-
Hyphal length (cm)	964a ± 198	921a ± 188	-	510a ± 169	366a ± 67	-
Number of spores	277a ± 76	196a ± 67	-	180a ± 58	122a ± 23	-
Root length (cm)	-	21a ± 2.2	21a ± 5.6	-	19a ± 4.4	15a ± 11
Root fresh weight (mg)	-	59a ± 11	55a ± 15	-	47a ± 4.5	50a ± 11

[a] N is the number of replicates for each treatment.

[b] Values (means ± SE) within the same row followed by an identical letter are not significantly different ($P \leq 0.05$).

Radiocaesium uptake and translocation by fungal hyphae, mycorrhizal roots and non-mycorrhizal roots

At the end of the experiment, 5.2, 33.3 and 32.8% of the ^{134}Cs initially supplied in the EC was taken up by the hyphae and/or roots in the HC, RHC and RC treatments, respectively (Table 2). This uptake was significantly lower in the HC treatment as compared to both the RHC and RC treatments, while this uptake did not significantly differ between the RHC and RC treatments ($P \leq 0.01$). The difference between uptake in the HC versus RHC and RC treatments was explained by the importance of ^{134}Cs uptake by the roots developing in the ECs. The ^{134}Cs activity measured in these roots, accounted for 78.1 and 82.3% of the total ^{134}Cs taken up by the hyphae and/or roots in the RHC and RC, respectively.

The translocation of ^{134}Cs from the ECs to the CC (i.e. the total ^{134}Cs content in the CC) accounted for 4.8, 7.2 and 5.8% of the ^{134}Cs initially supplied in the HC, RHC and RC treatments, respectively, but did not significantly differ among these treatments. Similarly, the ^{134}Cs content of the roots in the CC did not differ among the treatments even when ^{134}Cs root content was expressed in concentrations (Bq cm^{-1} or Bq mg^{-1} f. wt. of roots). However, the ^{134}Cs root content in the CC accounted for 80.8, 19.2 and 16.5% of the total ^{134}Cs taken up by the hyphae and/or roots in the HC, RHC and RC treatments, respectively. This relative content was significantly higher in the HC treatment as compared to both the RHC and RC treatments ($P \leq 0.01$), although the

root length or the root fresh weight in the CC was identical for these treatments as mentioned in Table 1.

Radiocaesium activity in the gel of the CC was also low (0.60, 0.72 and 0.40% of the initial ^{134}Cs supplied in the ECs of the HC, RHC and RC treatments, respectively).

In the Formaldehyde control treatment, trace amounts of ^{134}Cs were detected in the roots of the ECs treatments and in the root and gel of the CC, and no ^{134}Cs was detected in the hyphae of the EC treatments (data not shown). In the Control and Root-control treatments, no ^{134}Cs activity was detected.

Table 2 Radiocaesium activity in the culture compartment (CC) and the experimental compartments (ECs) for the hyphal compartment (HC) treatment, the mycorrhizal root and hyphal compartment (RHC) treatment and the root compartment (RC) treatment, without formaldehyde added to the solution in these compartments.

	HC N = 7[a]	**RHC N = 6**	**RC N = 6**
Cs activity content (Bq/Petri plate)			
Solution (EC's)	10135 (95) a[b]	7158 (67) b	7203 (67) b
Hyphae (EC's)	37 (0.35) a	19 (0.18) a	-
Root in the EC's	-	2783 (26) a	2878 (27) a
Gel with fungal biomass (CC)	64 (0.60) a	78 (0.72) a	43 (0.40) a
Roots in the CC	454 (4.2) a	692 (6.4) a	578 (5.4) a
Cs activity concentration (Bq/mg^{-1} FW)			
Solution (EC's)	ND[c]	ND	ND
Hyphae (EC's)	ND	ND	ND
Root in the EC's	-	49 a	60 a
Gel with fungal biomass (CC)	2 a	3 a	2 a
Roots in the CC	1.06 a	1.85 a	1.39 a
Cs activity concentration (Bq/cm)			
Solution (EC's)	-	-	-
Hyphae (EC's)	0.04 a	0.03 a	-
Root in the EC's	-	127 a	151 a
Gel with fungal biomass (CC)	ND	ND	ND
Roots in the CC	3.1 a	4.7 a	4.1 a

[a] N is the number of replicates for each treatment.
[b] Values in parentheses indicate percentages of the ^{134}Cs initially supplied in the external compartments (EC's). Values within the same row followed by an identical letter are not significantly different ($P \leq 0.05$).
[c] ND: not determined

^{134}Cs uptake, accumulation and translocation versus fungal hyphae, mycorrhizal and non-mycorrhizal roots' variables

For the HC treatment, the total uptake of ^{134}Cs was positively correlated with the length of hyphae in this EC ($r = 0.8196$ with $p = 0.024$). Root ^{134}Cs content, in the CC, was positively correlated with hyphae length in the HC and the number of hyphae crossing the

partition wall ($r = 0.7914$ with $p = 0.034$ and 0.7724 with $p = 0.042$, respectively), but no correlation was found between the AM fungal root colonization (*%F* and *%I*) in the CC and root ^{134}Cs content or concentrations (Bq cm^{-1} and Bq mg^{-1} f. wt. of root) in this compartment. The hyphae ^{134}Cs content in the HC was positively correlated with hyphal length ($r = 0.9021$ with $p = 0.005$) and with the number of spores ($r = 0.8246$ with $p = 0.022$) in this compartment.

For the RHC treatment, the total uptake of ^{134}Cs was neither correlated with the length of roots or hyphae in this EC. Likewise, no correlation was observed between the total uptake of ^{134}Cs and root ^{134}Cs content or concentrations (Bq cm^{-1} and Bq mg^{-1} f. wt. of root) in the CC and in the RHC. No correlation was found between the number of hyphae or roots crossing the partition wall between the EC and the CC and root ^{134}Cs content or concentrations in the CC. Similarly, no correlation was found between the root ^{134}Cs content of the RHC and the length of roots or the length of hyphae in this compartment. In the CC, a negative correlation was found between the root ^{134}Cs content in the CC and the *%F* ($r = -0.9163$ with $p = 0.010$) but not with the *%I*. The root ^{134}Cs content in the RHC was positively correlated with *%F* ($r = 0.5072$ with $p = 0.019$) but not with *%I*. The extraradical hyphae ^{134}Cs content in the RHC was positively correlated with hyphal length ($r = 0.8877$ with $p = 0.005$) but not with the number of spores in this compartment.

For the RC treatment, the total uptake of ^{134}Cs was correlated with the length of roots in this EC ($r = 0.8902$ with $r = 0.017$). The root ^{134}Cs content in the CC was positively correlated with the length of the roots in both the CC and the RC ($r = 0.9181$ with $p = 0.010$ and $r = 0.8629$ with $p = 0.027$, respectively), but was not correlated with the number of roots crossing the partition wall between the two compartments. The root ^{134}Cs content in the RC was positively correlated with the root length in this compartment ($r = 0.8284$ with $p = 0.042$). Finally, the root ^{134}Cs content or concentrations (Bq cm^{-1} and Bq mg^{-1} f. wt. of root) in the CC and the EC were not correlated.

Discussion

The monoxenic culture system with two compartments physically separated by a plastic partition wall (St Arnaud *et al*., 1995) has been used in several transport studies, involving essential nutrients such as phosphorus (Joner *et al*., 2000b and Nielsen *et al*., 2002) and nitrogen (Hawkins *et al*., 2000) as well as pollutants such as uranium (Rufyikiri *et al*., 2002 and Rufyikiri *et al*., 2003) and radiocaesium (Declerck *et al*., 2003). In these experiments, the fungal biomass developing in the labelled compartment was generally low and could, at least partly, be related to the apparent difficulty for the hyphae to cross the partition wall between the two compartments. This restricted hyphal development could have resulted in an underestimation of the effect of AM fungi on the uptake and translocation as suggested by

Rufyikiri *et al*. (2003). Recently, these authors improved the two-compartmented monoxenic culture system by extending the gelled medium 2 mm above the partition wall between the CC and EC. With this improvement, the number of hyphae crossing the partition between the two compartments was strongly augmented and, as a consequence, the fungal biomass developing in the EC was increased by 6-fold. In the present study, we followed the same protocol, but pre-mycorrhizal root apexes were used instead of non-mycorrhizal roots that had still to be inoculated with AM fungal propagules (Rufyikiri *et al*., 2002, Rufyikiri *et al*., 2003 and Declerck *et al*., 2003). Such pre-mycorrhized root apexes offered the advantage of rapid external hyphae development into the CC and vigorous root and hyphal growth throughout the experiment. Both adaptations of the monoxenic culture system resulted in a drastic increase in fungal biomass (7-fold) developing in the EC of the HC treatment as compared to a previous study conducted on radiocaesium uptake and translocation on the same MSR^{Cs} medium (Declerck *et al*., 2003). The consecutive enhanced fungal biomass developing in the EC, resulted in an increase in radiocaesium uptake by hyphae in the EC and translocation towards roots in the CC. Translocation from EC to CC accounted for 4.8% of the isotope added to the EC, which was nearly 5-fold higher than previously reported by Declerck *et al*. (2003). Translocation of radiocaesium to roots was further strongly correlated with the length of hyphae in the HC as shown by Declerck *et al*. (2003) and by the number of living hyphae connecting the HC and CC, as already observed for P (Nielsen *et al*., 2002). Such observations,

combined with the absence of translocation of radiocaesium in the formaldehyde control treatment, unambiguously support the capacity of hyphae to translocate radiocaesium. Moreover, they substantiate the importance of the active fungal biomass that connect the labelled compartment to the root and the density of the hyphae in contact with the radionuclide for radiocaesium translocation.

The total uptake of radiocaesium by the mycorrhizal roots and hyphae in the RHC treatment and by the roots alone in the RC treatment was higher than the total uptake by hyphae alone in the HC treatment. However, the radiocaesium taken up in the RHC and RC treatments mostly remained in the roots of these compartments. As a consequence, we observed that the relative translocation of radiocaesium by the hyphae developing in the HC treatment was higher than the relative translocation of radiocaesium by the mycorrhizal and non-mycorrhizal roots developing in the RHC and RC treatments. Thus the translocation/uptake ratio for AM fungal hyphae was higher than the one of the roots, mycorrhizal or not.

The mechanisms governing radiocaesium uptake and translocation by the AM fungi remain speculative as outlined by Declerck *et al*. (2003). Uptake of radiocaesium is probably similar to the uptake of K and is susceptible to be located, at least, partly at the level of the BAS, as it has been suggested for other elements (Bago, 2000). Translocation of radiocaesium might be channeled by motile and tubularly interconnected vacuoles as suspected for K in

ectomycorrhizal fungi (Orlovich and Ashford, 1993). These authors showed that P and K were associated in vacuoles in the extraradical hyphae and Jentschke *et al*. (2001) observed that long distance translocation of K was dependent on the concurrent movement of P in *Paxillus involutus*. Their occurrence and role in AM fungi have recently been documented (Ashford and Allaway, 2002, Burleigh and Jakobsen, 2002, Bago *et al*., 2002 and Uetake *et al*., 2002) and are believed to participate in long distance P translocation (Ezawa *et al*., 2002). The high degree of chemical similarity between K and radiocaesium (White and Broadley, 2000) supports a possible translocation of radiocaesium through the tubularly interconnected vacuole channel and merits further investigation, although other radiocaesium translocation mechanisms, such as cytoplasmic streaming per se, should not be neglected (Declerck *et al*., 2003).

The relatively low radiocaesium translocation of the roots in the RHC and RC treatments to the CC and its preferential accumulation in the roots in the RHC and RC, observed in this study are poorly understood. A possible explanation could be the absence of a strong sink driving force of the excised roots since radiocaesium is known to behave to a certain extent like K in plants (K concentrations are generally higher in leaves and in developing structures). The lack of the aerial part in the monoxenic culture system and the presence of root meristems could at least partially explain the overall low translocation of radiocaesium to the CC compartment, in the mycorrhizal and non-mycorrhizal roots. Richter and Marschner (1973)

showed that only 25% of the K taken up in the apical 3 cm of the roots is translocated to the shoot of corn plants. The results of our study are rather in accordance with this study since only 25.4 and 16% of the total uptake of radiocaesium by the roots in the ECs of the RHC and RC treatment were translocated to the CC, respectively. It should also be noted that the translocation of radiocaesium along the roots could have been unpaired due to the possible accumulation of radiocaesium in the root cells to maintain cell osmotic potential (Leigh and Wyn Jones, 1984). Indeed, the lack of K in the medium of the ECs might have resulted in its substitution by radiocaesium in the root cells for these functions and thus restricted radiocaesium transport towards the xylem. Another possible explanation may be the inhibition of the KOR (outward-rectifying potassium) channels also due to the lack of K in the medium of the ECs. The KOR channels mediate the majority of the efflux of K^+ and Cs^+ from the roots cells, but as no K^+ was provided in the external solution of the EC the probability of openness of the KOR channels was probably close to zero to prevent leakage of K and thus radiocaesium from the root cells (Maathuis and Sanders, 1997). Moreover, Pilot *et al*. (2003) have observed that in K-starved plants, the expression of genes encoding for a KOR channel in *Arabidopsis thaliana* was reduced. Consequently, following the influx of radiocaesium in root cells through the K^+/H^+ symporter or more likely through the VIC (voltage-insensitive cation) channels (Broadley *et al*., 2001), the limitation of the efflux of Cs^+ lead to its accumulation in the root cells (Zhu *et al*., 1999). Concurrently, the reduction of radiocaesium loading in the xylem resulted in the decrease of the

amount of Cs^+ translocated along the roots. For instance, Buysse *et al*. (1996) and Smolders *et al*. (1996) have observed that the root/shoot radiocaesium concentration ratio increased with decreasing K supply, thus supporting this hypothesis.

In addition, we observed that radiocaesium content in the mycorrhizal roots in the CC of the RHC treatment was negatively correlated to the frequency of root colonization in the CC, while a positive correlation was observed between radiocaesium content in mycorrhizal roots in the EC of the RHC treatment and the frequency of root colonization in this compartment. If we consider that the hyphae of the RHC treatment had the same translocation capacity as the hyphae of the HC treatment, we can estimate that the translocation of radiocaesium by the mycorrhizal roots alone of the RHC treatment (not taking account of the potential role of intraradical hyphae) was halved when compared with the radiocaesium translocation by the non-mycorrhizal roots of the RC treatment. These observations suggest that AM fungi might play a role in the local accumulation of radiocaesium in the roots and in its low translocation along them, therefore interfering with the normal accumulation/transport of radiocaesium. The modifications of the plasma membrane of the root cells surrounding intercellular hyphae may be associated to this hypothesis. The plasma membrane invaginates and proliferates around the hyphal branches of the forming arbuscules, increasing up to 10-fold its surface area (Alexander *et al*., 1989). Yet, the more stringent effect of the AM fungi on the plasma membrane is the regulation of

genes involved in the membrane transport processes (Rausch *et al*., 2001 and reviews by Burleigh and Bechmann, 2002 and Ferrol *et al*., 2002). Recently, Burleigh *et al*. (2003) showed that the expression of a Zn transporter was down-regulated in the roots of *Medicago truncatula* when colonized by AM fungi and was associated with a reduced level of Zn in both roots and leaves. Consequently, it is possible to envision that the translocation of radiocaesium within the roots could be perturbed by the presence of AM fungi due to the down-regulation of genes encoding transporters of radiocaesium at the level of the root cell plasma membrane. Such a process has been proposed by Burleigh and Harrison (1997) and Burleigh and Bechmann (2002) but still needs to be ascertained. The comparison of the expression of identified radiocaesium transporters in mycorrhizal roots and non-mycorrhizal roots to test whether AM fungi can modify their regulation and consequently the loading of radiocaesium in the xylem should now be conducted. Indeed, as suggested by White and Broadley (2000), the down-regulation of VIC or KOR channels might specifically restrict radiocaesium translocation to the shoot and offer a suitable solution for radiocaesium phytostabilisation.

Chapter III

Transport of radiocaesium by arbuscular mycorrhizal fungi to *Medicago truncatula* under *in vitro* conditions

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Transport of radiocaesium by arbuscular mycorrhizal fungi to *Medicago truncatula* under *in vitro* conditions

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Abstract

The capacity of arbuscular mycorrhizal (AM) fungi to take up and translocate radiocaesium (Cs) to their host has been shown using the root-organ culture (ROC) system. However, the absence of photosynthetic tissues, lack of a normal root hormonal balance and incomplete source–sink relationships may bias the bidirectional transfer of elements at the symbiotic interface and complicate transport studies. Accordingly, we developed a novel culture system [i.e. the Arbuscular Mycorrhizal-Plant (AM-P) *in vitro* culture system], where AM fungi and an autotrophic host plant develop under strict *in vitro* conditions. With this system, we unambiguously demonstrated the capacity of AM fungi to transport Cs. The extraradical fungal hyphae took up 21.0% of the initial supply of

^{134}Cs. Translocation to the plant represented 83.6% of the ^{134}Cs taken up. Distribution of ^{134}Cs in the host plant was 89.8% in the mycorrhizal roots and 10.2% in the shoot. These results confirm that AM fungi can take up, translocate and accumulate Cs. They further demonstrate unambiguously and for the first time that Cs can be transferred from AM fungi to host tissues. These results suggest a potential involvement of AM fungi in Cs biogeochemical cycle and in plant Cs accumulation.

Introduction

Mineral nutrition of arbuscular mycorrhizal (AM) plants has received tremendous attention in the past decades and continues to be the subject of intense research activities. However, if the transport of essential elements (e.g. phosphorus, nitrogen) by AM fungi is well documented, less consideration has been given to other nutrients or pollutants (Smith and Read, 1997; Leyval *et al*., 2002).

Radiocaesium (Cs) is the most threatening radioelement present in the environment due to its long half-life (30.2 years for ^{137}Cs), low mobility in soils (Delvaux *et al*., 2001) and chemical similarity to potassium (Bowen, 1979). It can be taken up by plants (White and Broadley, 2000) and enter the food chain (Coughtrey and Thorne, 1983). Studies on the biogeochemical cycle of Cs in forest ecosystems have clearly shown that ectomycorrhizal fungi can accumulate significant amounts of Cs both in their fruiting bodies and in below-

ground mycelium (Nikolova *et al*., 1997; Steiner *et al*., 2002). In contrast, mycorrhiza-mediated accumulation and transport of Cs remains uncertain or even controversial in ecosystems dominated by herbaceous plants, which are mainly colonized by AM fungi (Dupré de Boulois *et al*., 2005a). Earlier studies suggested that the accumulation of Cs in mycorrhizal plants was either reduced (Dighton and Terry, 1996; Berreck and Haselwandter, 2001), similar (Rogers and Williams, 1986; Rosén *et al*., 2005) or increased (McGraw *et al*., 1979; Dighton and Terry, 1996; Entry *et al*., 1999; Rosén *et al*., 2005) as compared with non-mycorrhizal plants, depending on the plant and AM fungal species considered. However, these studies did not assess fungal transport or immobilization but rather the combined effect of AM fungi and root uptake on plant Cs accumulation (Joner *et al*., 2004). To overcome this limitation, compartmented pot systems were subsequently developed to allow the AM extraradical hyphae to develop in a labelled compartment from which the host roots were physically excluded (see review by Schweiger and Jakobsen, 2000). Such approaches were used to determine whether the AM fungal mycelium was able to transport rubidium (an analogue of K and Cs – Suzuki *et al*., 2001) and Cs (Joner *et al*., 2004). However, these studies also led to contradictory results. Indeed, while Suzuki and colleagues (2001) demonstrated that AM fungi could transport Rb, Joner and colleagues (2004) observed that Cs was not even translocated towards plant roots.

The inconsistency between these experiments is probably related to the influence of soil constituents and of K on bioavailability of Cs

(Delvaux *et al*., 2001), root uptake of Cs (Hampton *et al*., 2005) and plant accumulation of Cs (Zhu *et al*., 2000). Furthermore, transport of Cs by AM fungi, and especially uptake at the level of K/Cs transporters, is probably also influenced by K as hypothesized by Dupré de Boulois and colleagues (2005a). In addition to these physiological aspects, the sterility in the compartmented pot systems could not be guaranteed and the impact of undesirable microorganisms on the bioavailability of Cs could therefore not be excluded.

To avoid these drawbacks, *in vitro* root-organ cultures (ROC) using bicompartment Petri plates were developed to study element transport by AM fungi (Rufyikiri *et al*., 2005). Using these systems, the indisputable role of extraradical mycelium in the uptake and translocation of Cs was demonstrated (Declerck *et al*., 2003; Dupré de Boulois *et al*., 2005b). However, these ROC systems suffered from the absence of photosynthetic tissues, and were thus inadequate to show whether Cs was transferred from the intraradical fungal structures to the plant tissues. Moreover, the presence of sucrose in the culture medium, lack of a normal root hormonal balance and incomplete source–sink relationships may have altered plant–fungal symbiotic interactions including bidirectional exchanges (Fortin *et al*., 2002). For these reasons, a culture system for the *in vitro* mycorrhization of autotrophic plants was recently developed by Voets and colleagues (2005). This system associated roots of micropropagated plantlets to an AM fungus under *in vitro* conditions, while the shoots developed under open-air conditions. This system allowed the production of an

abundant active mycelium bearing several thousands of spores. However, the development of the shoot in open-air conditions could not exclude biotic and abiotic stresses among which the potential systemic infection by various microorganisms.

In the present study, we developed a compartmented culture system in which both an AM fungus and an autotrophic host plant developed under strict *in vitro* conditions. This allowed us to determine whether AM fungi are able to transport Cs to their host plant.

Experimental Procedures

Arbuscular Mycorrhizal-Plant (AM-P) in vitro culture system

The Arbuscular Mycorrhizal-Plant (AM-P) *in vitro* culture system (Fig. 1) consisted of three compartments: a shoot compartment (SC) where the shoot developed, an RC where roots and AM fungi developed on a synthetic solid medium and an HC where only the AM extraradical mycelium developed in a synthetic liquid medium. The RC was physically separated from the HC by a plastic wall. The hyphae in the RC proliferated in the HC by crossing this plastic wall, while the roots were trimmed to leave the HC void of roots.

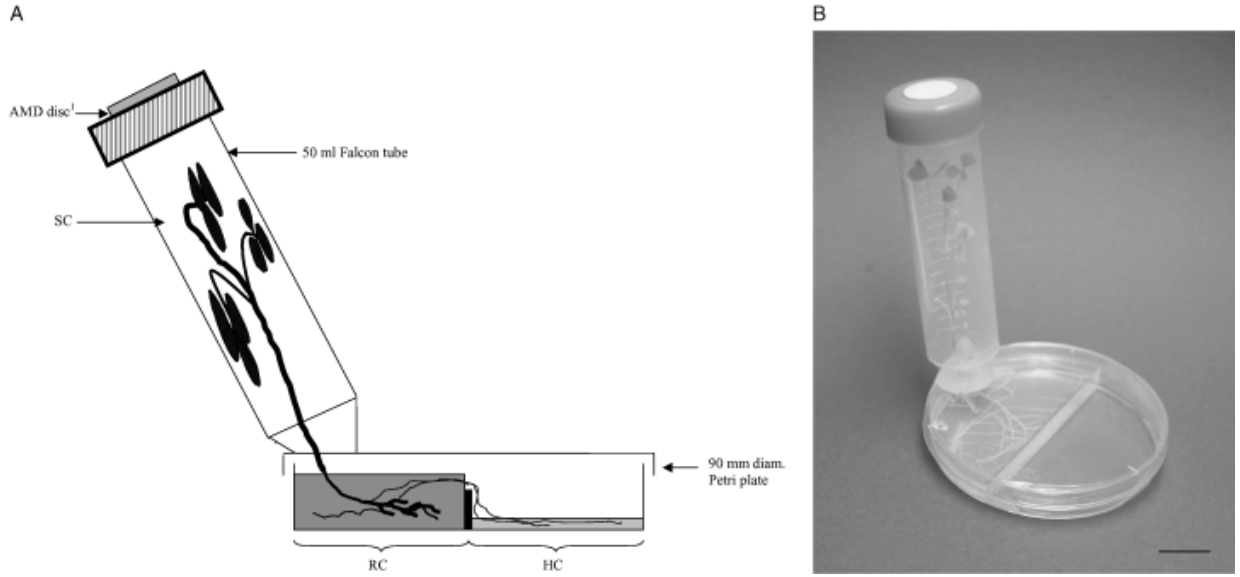
The set-up of each AM-P *in vitro* culture system was achieved as follows. A hole (5 mm diameter) was made in the lid of the Petri plate and the lower part of a Falcon tube (50 ml, Sarstedt Aktiengesellschaft

and Co, Nümbrecht, Germany) was cut diagonally (hole of about 5 mm diameter) to obtain an angle of 65 ± 5° when placed on the lid of the Petri plate. The tube and the Petri plate were then glued together using plastic thermofusible glue (Metabowerke GmbH, Nürtingen, Germany). A hole (10 mm diameter) was made in the cap of the Falcon tube, and a filter (18.6 mm diameter with inner efficient gas exchange capacity of 10 mm diameter and surface of 78.5 mm^2) was fixed on the cap. The filter used was Adhesive Microfiltration Disc (AMD) in polypropylene laminated PTFE (Tissue Quick Plant Laboratory, Hampshire, UK) which prevented microbial contamination but allowed gas exchanges [Nominal Pore Size (NPS) of 0.25 µm]. Each AM-P *in vitro* culture system was then sterilized at 25 kGy by gamma irradiation.

Biological material

A *Glomus* sp. [formerly characterized as *Glomus lamellosum* Dalpé, Koske and Tews (MUCL 43195)] grown in association with Ri T-DNA transformed carrot (*Daucus carota* L.) roots was purchased from GINCO (http://www.mbla.ucl.ac.be/ginco-bel) and supplied in Petri plates (90 mm diameter) on the MSR medium (Declerck *et al*., 1998 modified from Strullu and Romand, 1986), but solidified with 3 g l^{-1} GelGro™ (ICN, Biomedicals, Irvine, CA, USA). Petri plates were incubated in an inverted position in the dark at 27°C. Several thousand spores were produced within a period of 5 months

Fig. 1. The Arbuscular Mycorrhizal-Plant (AM-P) *in vitro* culture system. (A) schematic representation of the AM-P *in vitro* culture system and (B) photograph of the AM-P *in vitro* culture system with a 1-month-old culture of *M. truncatula* L., cv. Séjalong associated to *Glomus* sp. (MUCL 43195). Scale bar = 20 mm. The system allows the spatial separation of a shoot compartment (SC) where the stem and leaves of a plant develop, a root compartment (RC) where roots and hyphae of an AM fungus grow and a hyphal compartment (HC) in which only the hyphae are allowed to proliferate. [1]Adhesive Microfiltration Disc (AMD) in polypropylene laminated PTFE avoiding contamination but allowing gas exchanges.



Seeds of *M. truncatula* L., cv. Séjalong were surface-sterilized by immersion in sodium hypochlorite (8%) for 10 min, rinsed in deionized sterile water and germinated in Petri plates (90 mm diameter) on 40 ml of MSR^{Cs} medium (Declerck *et al*., 2003) lacking sucrose and vitamins and solidified with 4 g l^{-1} agar fine powder (Aldrich agar fine powder, Sigma Aldrich Chemie, GmbH, Steinheim, Germany). Germination was conducted in a controlled environment chamber at 20°C in the dark for 10 days.

Experimental design

Ten-day-old *M. truncatula* plantlets with shoots of approximately 50 mm long and primary roots of approximately 70 mm long, and presenting a range of two to five secondary roots, were selected for transfer in the AM-P *in vitro* culture systems. Shoots of *M. truncatula* plantlets were inserted through the opening between the RC and SC. The roots were placed in the RC on the surface of the solid MSR^{Cs} medium (Declerck *et al*., 2003) lacking vitamins and sucrose. The RC was filled with 33.5 ml of the MSR^{Cs} solidified with 4 g l^{-1} agar fine powder following the protocol of Dupré de Boulois and colleagues (2005b) so that the medium extended 2 mm above the partition wall. Two holes of 1.75 cm^3 were then made with a cork borer in the MSR^{Cs} medium (15 mm from the partition wall and edge of the Petri plate) to facilitate addition of medium during the time-course of the experiment to insure adequate plant and AM fungal growth (see below). The initial volume of MSR^{Cs} medium in this

compartment was therefore 30 ml and the initial amount of K in this compartment was 0.21 mg for a concentration of 0.18 mM.

Roots of *M. truncatula* plantlets were subsequently inoculated with the ROC-produced spores (± 100) of *Glomus* sp. following the method described by Declerck and colleagues (2003). The bicompartment Petri plates of the AM-P *in vitro* culture systems were then sealed using Parafilm (Pechiney, Menasha, WI, USA) and wrapped in black plastic bags to maintain roots and *Glomus* sp. in the dark. The AM-P *in vitro* culture systems were subsequently incubated in a controlled environment chamber (20°C, 16 h photoperiod). Daylight lamps provided an average photosynthetic photon flux at the level of the SCs of 225 µmol m^{-2} s^{-1}.

Two weeks after inoculation, the AM-P *in vitro* culture systems were supplied weekly with 0.25 ml of MSR^{Cs} medium (concentrated 10 times) in each hole made in the medium up to 2 weeks before radio-isotopic labelling (10 weeks after inoculation). The plants therefore received throughout the experiment a total of 0.46 mg of K.

Three weeks after inoculation, AM fungal mycelium started to contact the roots and to develop in the entire volume of the RCs. Eight weeks after inoculation, the HCs were filled with 20 ml of liquid MSR^{Cs} medium, lacking sucrose, vitamins and KCl (no K was present in this compartment to prevent any possible competition between K and Cs for uptake) to ensure mycelium crossing the partition wall

between the RCs and HCs to develop in the HCs. Therefore, mycelium growth in the HCs was initiated only 2 weeks before radio-isotopic labelling.

Radio-isotopic labeling

Ten weeks after the beginning of the experiment (i.e. 2 weeks after initiation of hyphal development in the HCs), the liquid medium contained in the HCs was removed with a sterile syringe and immediately replaced by 5 ml of a fresh liquid MSR^{Cs} medium also lacking sucrose, vitamins and KCl. Filter-sterilized (Acrodisc® Syringe Filters, PALL Corporation, Ann Arbor, MI, USA) radiocaesium (^{134}Cs, Cs treatment, eight replicates) was added to the liquid medium of the AM-P *in vitro* culture systems. The concentration of ^{134}Cs in the medium was 1675 Bq ml^{-1}. The source of ^{134}Cs was in form of $CsNO_3$ in water, supplied by the laboratory of nuclear chemistry (CMAT, UCL, Belgium).

Four control treatments were integrated. The first two were included to measure the photosynthetic activity of either MYC (eight replicates) or N-MYC (eight replicates) plants in the AM-P *in vitro* culture system. The other two aimed at assessing the transport capacity of *Glomus* sp. in the AM-P *in vitro* culture system by using a radio-tracer of P (P treatment, eight replicates) and determining whether the translocation of ^{33}P from the HCs to the RCs was mediated by active processes involving AM fungal hyphae by using

formaldehyde (F treatment, four replicates). For the P treatment filter-sterilized (Acrodisc® Syringe Filters, PALL Corporation, Ann Arbor, MI, USA) phosphorus (^{33}P) was added to the liquid medium of AM-P *in vitro* culture systems. The concentration of ^{33}P in the medium was 1395 Bq ml^{-1}. The source of ^{33}P was orthophosphate in dilute hydrochloric acid (< 0.1 M) supplied by Amersham Pharmacia Biotech (Buckinghamshire, UK). For the F treatment, formaldehyde was added in the HCs following the description of Dupré de Boulois and colleagues (2005b).

Harvest and plant – AM fungal analyses

At the end of the experiment (i.e. 2 weeks after the addition of ^{134}Cs), the total extraradical hyphal length, root length and number of spores in the RCs and HCs of the Cs treatment were estimated. The extraradical hyphae and root length were determined as detailed in Declerck and colleagues (2003), while spore count followed the methodology described in Declerck and colleagues (2001b). The number of total and active hyphae (i.e. presenting bidirectional flux of cytoplasm/protoplasm) on the partition wall was measured under dissecting microscope [×40 – Olympus SZ40, Olympus Optical (Europa) Gmbh, Germany].

The shoots of *M. truncatula* were then collected by cutting the shoots at the level of the solidified MSR^{Cs} medium contained in the RCs. The liquid medium in the HCs was sampled and the HCs were

rinsed twice with 5 ml of distilled water for ^{134}Cs activity measurements. Fungal mycelium developing into the HCs was then collected. In the RCs, roots were removed from the solidified MSR^{Cs} medium and cleaned-free from the remaining gel and extraradical mycelium. The solidified MSR^{Cs} medium containing extraradical mycelium was then collected.

Shoot fresh weight and root fresh weight of the plants were measured. Samples were then subjected to ^{134}Cs counting on a Wallac 1480 Gamma Counter (Wallac, Turku, Finland).

Arbuscular mycorrhizal fungal root colonization was observed under compound microscope [×100 to ×400 – Olympus BH2, Olympus Optical (Europa) Gmbh, Germany] following clearing with 10% KOH and staining with 0.2% Trypan blue (Phillips and Hayman, 1970). Root colonization was assessed by the evaluation of frequency (%F) and intensity (%I) of AM fungal colonization (Plenchette and Morel, 1996). Arbuscular (%A), vesicular (%V) and hyphal (%H) colonization was assessed (McGonigle *et al*., 1990).

Photosynthetic activity of the mycorrhizal and non-mycorrhizal plants of the MYC and N-MYC treatments was measured by determining the NPR using Infra-Red Gas Analyser (IRGA), model LCA-4 (ADC Bioscientific, UK).

Transport of ^{33}P was assessed by measuring ^{33}P activities in the liquid medium and fungal biomass in the HCs, solid medium containing fungal biomass in the RCs and roots and shoots of *M. truncatula*. Prior to ^{33}P counting, fungal and plant samples were dissolved in Soluene 350 (Packard BioScience, Groningen, the Netherlands) and then cleared using H_2O_2 (30% v/v). Liquid scintillation cocktail (Ultima Gold™, Packard BioScience, Groningen, the Netherlands) was then added to all samples in 10 ml aliquots. Samples were then subjected to ^{33}P counting on a Packard TR2500 Liquid Scintillation Analyser (Packard Instrument, Meriden, CT, USA).

Results

Plant and AM fungal growth variables in the AM-P in vitro culture system

Plant and AM fungal growth variables of the Cs treatment are presented in Table 1. At the end of the experiment, *M. truncatula* plants were still actively growing. Numerous fine and white roots, as well as green leaves and production of foliar primordia were observed in each experimental unit. In both the RCs and HCs, AM fungal mycelium developed profusely. In the RCs, the extraradical mycelium was characterized by runner hyphae producing branched absorbing structures (BAS) at regular intervals. Anastomoses were also observed along runner and lower order hyphae. Several hundreds of newly

produced spores were counted, many of which were developing in BAS-spore configurations. In the HCs, runner hyphae were predominant. BAS were also observed but were less abundant with fewer branches and were not produced at regular intervals. Spores were observed isolated or associated to BAS. Root colonization in the RCs was evidenced by the presence of hyphae, arbuscules and vesicles.

Table 1 Plant and arbuscular mycorrhizal fungal growth variables in the Cs treatment, estimated by the shoot fresh weight (SFW), root fresh weight (RFW), root length, hyphal length, number of spores and by the frequency (%F) and intensity (%I) of fungal colonization, arbuscules (%A), vesicles (%V) and intraradical hyphae (%H) in the shoot compartment (SC), root compartment (RC) and hyphal compartment (HC).

SC	SFW (mg)	509 ± 62[a]
RC	RFW (mg)	877 ± 102
	Root length (cm)	481 ± 54
	Hyphal length (cm)	1047 ± 622
	Number of spores	1613 ± 1048
	%F	55 ± 28
	%I	32 ± 12
	%A	23 ± 18
	%V	11 ± 10
	%H	13 ± 5
HC	Hyphal length (cm)	175 ± 102
	Number of spores	19 ± 28
SC and RC	Plant fresh weight (mg)	1386 ± 111
RC and HC	Number of active crossing hyphae[b]	84 ± 58
	Total number of crossing hyphae[c]	96 ± 66

a. Values correspond to the means ± the standard errors (SE) of eight replicates.
b. The number of active hyphae at the end of the labelling period.
c. The number of active and inactive hyphae at the end of the labelling period.

Numerous hyphae were observed to cross the partition wall separating the RCs from the HCs, within the 2-weeks labelling period. The percentage active hyphae measured at that time represented 87.5% of the total hyphae crossing the partition wall.

The photosynthetic activity of *M. truncatula* was measured in the AM-P *in vitro* culture system of the MYC and N-MYC treatments. The NPR were 5.66 and 5.48 µmol m^{-2} s^{-1} for the MYC and N-MYC plants, respectively, and did not significantly differ between these treatments.

Phosphorus transport in the AM-P in vitro culture system

^{33}P supplied to the HCs of the P treatment was taken up by the extraradical mycelium developing in the HCs, translocated towards the mycorrhizal roots developing in the RCs and transferred to the plant tissues. Uptake by the extraradical mycelium developing in the HCs accounted for 98.3% of the initial supply of ^{33}P in the HCs. An average of 69.5% of the initial ^{33}P supplied in the HCs was measured in the roots of *M. truncatula* developing in the RCs. Translocation to the plant represented 91.7% of the ^{33}P taken up by the AM fungus, the remaining being located in the medium and in the fungal biomass of the RCs and in the fungal biomass of the HCs. Shoots of *M. truncatula* contained 20.7% of the initial ^{33}P supplied in the HCs. The distribution of ^{33}P in *M. truncatula* was 76.9% in the roots and 23.1% in the shoot.

The metabolic activity of the AM fungal mycelium developing in the HCs was stopped almost immediately after addition of formaldehyde. This was evidenced by the absence of cytoplasmic/protoplasmic flux in hyphae crossing the partition wall. Trace amounts of ^{33}P were detected in the roots of *M. truncatula*, fungal biomass of the HCs and in the solidified modified Strullu-Romand medium adapted for Cs studies (MSR^{Cs}) containing also fungal biomass of the RCs, but accounted for less than 0.01% of the initial supply of ^{33}P in the HCs. No ^{33}P was detected in the shoots of *M. truncatula*.

Radiocaesium transport in the AM-P in vitro culture system

^{134}Cs supplied to the HCs was taken up by the extraradical mycelium developing in the HCs, translocated towards the mycorrhizal roots developing in the RCs and transferred to the plant tissues (Table 2).

Table 2 Radiocaesium activities (Bq Petri plate^{-1}) and specific activities [Bq cm^{-1} and Bq mg^{-1} fresh weight (FW)] of the shoot in the shoot compartment (SC), roots, solid MSR^{Cs} medium and fungal biomass in the root compartment (RC) and fungal biomass, liquid MSR^{Cs} medium in the hyphal compartment (HC).

Activities (Bq)		
SC	Shoot	148 (1.8 ± 1.1)[a]
RC	Roots	1300 (15.9 ± 8.4)
	Solid MSR^{Cs} medium with fungal biomass	262 (3.2 ± 3.0)
HC	Fungal biomass	8.6 (0.1 ± 0.1)
	Liquid MSR^{Cs} medium	6656 (79.0 ± 9.9)
Specific activities (Bq cm^{-1})		
RC	Root	3.4 ± 1.9[b]
HC	Fungal hyphae	0.1 ± 0.1
Specific activity (Bq mg^{-1} FW)		
SC	Shoot	0.3 ± 0.2
RC	Roots	1.5 ± 0.7

a. Values in parentheses correspond to the percentages ± the standard errors (SE) of the ^{134}Cs initially supplied in the HCs from eight replicates.
b. Values correspond to the means ± the standard errors (SE) of eight replicates.

Of the initial supply of ^{134}Cs, 21.0% was taken up by the mycelium developing in the HCs. An average of 15.9% of the initial ^{134}Cs supplied in the HCs was measured in the roots of *M. truncatula* developing in the RCs. And 1.8% of the initial ^{134}Cs supplied in the HCs was found in the shoots of *M. truncatula*. Up to 89.8% of the plant ^{134}Cs content was located in the roots, and therefore 10.2% in the shoot. Overall, 17.7% of the initial ^{134}Cs supplied in the HCs was successively taken up by the AM fungus and translocated to the host plant. Translocation to the plant represented 83.6% of the ^{134}Cs taken up by the AM fungus, the remaining being located in the medium and in the fungal biomass of the RCs and in the fungal biomass of the

HCs. In the HCs, 0.1% of the initial ^{134}Cs supplied in the HCs was measured in the fungal biomass. Activities recorded in the solid MSR^{Cs}, containing also the AM extraradical fungal biomass of the RCs, represented 3.2% of the initial ^{134}Cs supplied in the HCs. Therefore, at the most, 16.4% of the ^{134}Cs taken up by the AM fungus in the HCs was immobilized by the fungal biomass.

Discussion

Adequacy of the AM-P in vitro culture system to study transport of radiocaesium

In the present study, we developed a strict *in vitro* autotrophic culture system in which an AM fungus was associated to the roots of *M. truncatula*.

The composition of the MSR^{Cs} medium was similar to the MSR^{Cs} medium used for ROC to study Cs transport (Declerck *et al*., 2003). Plants were photosynthetically active, as shown by the values of NPR measured in the MYC and N-MYC treatments. This allowed sucrose and vitamins to be excluded from the medium as in the study of Voets and colleagues (2005). The relatively high root colonization and formation of an extensive extraradical mycelium network bearing several hundred spores suggested that the C compounds, produced by the autotrophic *M. truncatula* plants, were transferred from the host to the AM fungi due to the obligatory C biotrophic nature of these

microorganisms (Smith and Read, 1997). Identically, P, which is the major nutrient transported by AM fungi to their host (Pearson and Jakobsen, 1993; Smith and Read, 1997), was measured in the roots and shoots of *M. truncatula*. The distribution of transported P within the mycorrhizal plants was similar to that observed by several authors under *in vivo* conditions (e.g. Cooper and Tinker, 1978; Smith *et al*., 2000) and consistent with P allocation in P-deficient plants (Schachtman *et al*., 1998). This indicates that the AM fungus was able to transport this element and thus to participate in the mineral nutrition of its host. The bidirectional exchange of photosynthates and P convincingly demonstrates the suitability of the AM-P *in vitro* culture system to study the transport of essential elements and pollutants by AM fungi.

Transport of Cs

In the present study, the transport of Cs by AM fungi was unambiguously demonstrated and thus indicates that AM fungi have the capacity to influence Cs accumulation by plants. This result was obtained with a low K^+ concentration in the RCs and the absence of K^+ in the HCs (see Declerck *et al*., 2003; Dupré de Boulois *et al*., 2005b) to preclude the probable influence of K on Cs transport and plant Cs accumulation (Zhu *et al*., 2000; Declerck *et al*., 2003; Dupré de Boulois *et al*., 2005b). As such, the results of this study are in contrast to the ones obtained by Joner and colleagues (2004) from compartmented pot experiments, who reported the absence of Cs

transport by AM fungi to plants. However, in their study, the 'K pool' available to the AM fungi and plant roots was high and has probably influenced both the capacity of the AM fungi to transport Cs and of the hosts to accumulate Cs. Indeed, even if the authors noticed that Cs bioavailability was likely to be high due to the K added to their substrates in both RCs and HCs, the influence of K on AM fungal Cs transport and plant Cs accumulation was not considered in their study.

The total uptake of Cs by the extraradical mycelium of the AM fungus represented 21% of the initial Cs supplied to the HC. This uptake was fourfold higher than in a study of Dupré de Boulois and colleagues (2005b) and 14-fold higher than in the one of Declerck and colleagues (2003). This higher uptake could be due to (i) the higher number of hyphae crossing the partition wall (Dupré de Boulois *et al*., 2005b), (ii) the higher biomass of the host [threefold higher in comparison with the study of Dupré de Boulois and colleagues (2005b)], representing a higher sink-force for K/Cs, (iii) the unperturbed bidirectional exchanges between AM fungi and host, i.e. no influence of the addition of sucrose in the culture medium, lack of a normal root hormonal balance and incomplete source–sink relationships (Fortin *et al*., 2002) and/or (iv) the presence of the shoot, acting as a new sink of K/Cs.

Mechanisms governing this uptake remain speculative. It is probable that the uptake of Cs was mediated by K transporters as observed for plants, bacteria and other fungi (Rodríguez-Navarro,

2000; White and Broadley, 2000). In fungi, two types of K/Cs transporters coexist (Rodríguez-Navarro, 2000). The high-affinity potassium (HAK) transporters (1 µM K^+K_m) mediate influx of Cs^+ with low selectivity between K^+ and Cs^+ (Sacchi *et al.*, 1997; Rodríguez-Navarro, 2000) and their expression is increased markedly at low external K concentrations ($[K^+_{ext}]$) (Santa-María *et al.*, 1997; Hampton *et al.*, 2004). The potassium TRK transporters are responsible for the influx of K/Cs in fungi at higher $[K^+_{ext}]$ (Rodríguez-Navarro, 2000). These TRK transporters, which also mediate active K/Cs influx, have a millimolar K^+K_m when $[K^+_{ext}]$ is millimolar but their K^+K_m can also decrease continuously down to 0.02 mM under restricted K^+ supply (Haro and Rodríguez-Navarro, 2002). The influx of Cs^+ by these transporters is greatly reduced by increasing $[K^+_{ext}]$ as K^+ is a competitive inhibitor of Cs^+ (Gassmann *et al.*, 1996). This suggests that at low $[K^+_{ext}]$, Cs^+ could be actively taken up by AM fungi, but that increasing $[K^+_{ext}]$ would lead to a rapid decrease of Cs^+ uptake. Further studies should be conducted on these transporters and their regulations in AM fungi to confirm that they may indeed mediate influx of Cs. In addition, the occurrence of the HAK transporters should be verified in AM fungi as they are not found in all fungal species in contrast to the TRK transporters (Durell *et al.*, 1999; Rodríguez-Navarro, 2000).

Following uptake, Cs^+ was translocated from the extraradical to the intraradical structures of the AM fungus. This translocation represented 84.3% of the Cs taken up by the fungus. This confirmed

that immobilization and/or adsorption of Cs by the extraradical mycelium is limited (Dupré de Boulois *et al*., 2005b). According to Declerck and colleagues (2003), Cs translocation could be mediated by the motile and tubularly interconnected vacuoles responsible for the long-distance translocation of P and K (Smith and Read, 1997; Jentschke *et al*., 2001). Following translocation, it can be suspected that Cs could accumulate in intraradical fungal hyphae before its efflux towards the interfacial apoplast. Indeed, K accumulation was previously observed in intraradical fungal hyphae and could result from the accumulation of P to maintain osmosis (Ezawa *et al*., 2002; Ryan *et al*., 2003). Potential compartmentalization of Cs in vesicles could also occur, in a similar manner as accumulation of K, but also of other elements such as U, Mn, Cu and Ni, has been observed in AM species producing these intraradical structures (Kaldorf *et al*., 1999; Weiersbye *et al*., 1999).

The transfer of Cs from AM fungi to plants was demonstrated for the first time in this study as 1.8% (i.e. 10.2% of the Cs translocated by the AM fungi to the host roots) of the initial Cs supplied in the HC was measured in the shoot of *M. truncatula*. However, in our experimental conditions, the amount of Cs actually transferred could not be determined because the Cs contained in the fungal intraradical structures and in the root tissues could not be differentiated. It can be assumed that the bioavailability of K and K nutritional status of AM fungi and their host is probably important in

Cs transfer as both expression and activity of plant and probably fungal K/Cs transporters can be affected by these factors.

In conclusion, this study demonstrated that AM fungi can transport Cs to their host plants, using a complete *in vitro* autotrophic culture system. It is the first direct observation that AM fungi may participate in plant Cs accumulation. This transport should further be investigated under varying levels of K in the medium, as this element has been shown to impact uptake and transport of Cs in plants (White and Broadley, 2000) and fungi (Terada *et al*., 1998), and considering different AM fungi–host combinations. In addition, the role of AM fungi on the translocation of Cs from root to shoot remains to be explored. In particular, the role AM fungi on the efflux of Cs into the xylem needs to be investigated (Dupré de Boulois *et al*., 2005b). Indeed, abscisic acid (ABA), which is present in higher concentrations in mycorrhizal roots (Danneberg *et al*., 1993; Ludwig-Müller, 2000; Meixner *et al*., 2005), was shown to restrict K/Cs efflux to the xylem (Gaymard *et al*., 1998; Tester, 1999; Pilot *et al*., 2003) and could therefore limit the translocation of Cs from the root to the shoot.

CHAPTER IV

Effect of K and P on the transport of Cs by arbuscular mycorrhizal fungi

A preliminary study

This work has been partially presented at:

The 5th International Conference on Mycorrhiza

Effect of K and P on the transport of Cs by arbuscular mycorrhizal fungi

H. Dupré de Boulois, S. Declerck

Abstract

Radiocaesium (Cs) is the most threatening radionuclide present in the environment. After its deposition on soil, following aboveground nuclear testing and catastrophic events such as the Chernobyl accident, it can be taken up by plants and fungi and thus enter the human food chain. Using *in vitro* culture systems, it was unambiguously demonstrated that arbuscular mycorrhizal (AM) fungi could take up, translocate and transfer Cs to their hosts, while Cs accumulation in the extraradical mycelium was limited. It was also suggested that potassium (K), a chemical analog of Cs, and phosphorus (P) could influence Cs transport by AM fungi. The purpose of this study was therefore to determine the effect of K and P on Cs transport by AM fungi. An *in vitro* compartmented culture sytem, associating an AM fungus and an autotrophic plant was used.

K and P were applied at several concentrations (0, 1, 10mM of K, and 30, 3000µM of P) in a compartment, also containing Cs, where only the AM hyphae could develop. The results showed that K and P could influence the transport of Cs by AM fungi, but not at all the concentrations tested. K reduced Cs transport by AM fungi when P was supplied at 30µM but not at 3000µM. P increased Cs transport at all the concentration of K tested. The mechanisms involved could not be resolved due to the experimental design used. The results, nevertheless, suggested that Cs could be taken up by AM fungi, at least partially, by transporters others than those functional in the uptake of K.

Introduction

Uptake, translocation and transfer of radiocaesium (Cs) by AM fungi has been demonstrated by Dupré de Boulois *et al*. (2006a) in an *in vitro* culture system where both AM fungi and an autotrophic plant were grown in association. In their experiment, and the ones of Declerck *et al*. (2003) and Dupré de Boulois *et al*. (2005b), the media used were depleted in potassium (K). Indeed, these authors suspected that K, as being chemically similar to Cs, could influence the transport of Cs as these elements are probably taken up, translocated and transferred by the same process. It was therefore suggested that K and Cs could compete for transport in AM fungi. This assertion could be supported by the results of Joner *et al*. (2004) who observed no

transport of Cs by AM fungi when K was supplied to the plant and AM fungi. Furthermore, Terada *et al*. (1998) showed a decreased Cs uptake and accumulation in fungi with increasing concentration of K. However, Baeza *et al*. (2005) found that there was probably no competition between K and Cs for uptake in a saprophyte fungus (*Pleurotus eryngii*) and an ectomycorrhizal fungus (*Hebeloma cylindrosporum*). Indeed, they found no correlation between increasing concentration of K in the medium and Cs uptake, thus confirming previous observations made by Yoshida & Muramatsu (1998).

Phosphorus (P) could possibly also influence Cs transport by AM fungi, as K is probably associated to P transport processes in AM fungi (Smith & Read, 1997) to maintain osmotic balance. In particular, K could be associated to P during translocation from the extraradical to the intraradical phase of the AM fungi. Indeed, P is believed to be translocated into a tubular vacuolar system in the form of polyphosphates (Ezawa *et al*., 2002). These molecules are charged negatively and are supposed to be associated to cations such as K and Mg (Orlovich & Ashford, 1993; Cole *et al*., 1998; Jentschke *et al*., 2001; Ryan *et al*., 2003). Therefore, it is possible that the translocation of Cs could be linked to the translocation of P. This implies that the transport of P could increase the one of Cs.

The purpose of this preliminary study was therefore to determine whether K competes with Cs for uptake and to investigate the

potential influence of P on Cs transport by AM fungi.

Experimental procedures

Biological material

A *Glomus* sp. [formerly characterized as *Glomus lamellosum* Dalpé, Koske and Tews (MUCL 43195)] grown in association with Ri T-DNA transformed carrot (*Daucus carota* L.) roots was purchased from GINCO (http://www.mbla.ucl.ac.be/ginco-bel) and supplied in Petri plates (90 mm diameter) on the MSR medium (Declerck *et al*., 1998 modified from Strullu and Romand, 1986), but solidified with 3 g l^{-1} GelGro™ (ICN, Biomedicals, Irvine, CA, USA). Petri plates were incubated in an inverted position in the dark at 27°C. Several thousand spores were produced within a period of 5 months.

Seeds of *M. truncatula* L., cv. Séjalong were surface-sterilized by immersion in sodium hypochlorite (8%) for 10 min, rinsed in deionized sterile water and germinated in Petri plates (90 mm diameter) on 40 ml of MSR^{Cs} medium (Declerck *et al*., 2003) lacking sucrose and vitamins and solidified with 4 g l^{-1} agar fine powder (Aldrich agar fine powder, Sigma Aldrich Chemie, GmbH, Steinheim, Germany). Germination was conducted in a controlled environment chamber at 20°C in the dark for 10 days.

Experimental design

Ten days old *M. truncatula* plantlets with shoots of approximately 50 mm long and primary roots of approximately 70 mm long, and presenting between two to five secondary roots, were selected for transplantation to the arbuscular mycorrhizal-plant (AM-P) *in vitro* culture systems described by Dupré de Boulois *et al*. (accepted). Briefly, the AM-P *in vitro* culture systems consists of three compartments: a shoot compartment (SC) where the shoot develop, a root compartment (RC) where roots and AM fungi develop on a synthetic solid medium and a hyphal compartment (HC) where only the AM extraradical mycelium develop in a synthetic liquid medium. The RC is physically separated from the HC by a plastic wall. The hyphae in the RC proliferate in the HC by crossing this plastic wall, while the roots are trimmed to leave the HC void of roots.

Shoots of *M. truncatula* plantlets were inserted through the opening between the RC and SC. The roots were placed in the RC on the surface of the solid MSR^{Cs} medium (Declerck *et al*., 2003) lacking vitamins and sucrose. The RC was filled with 20 ml of the MSR^{Cs} solidified with 4 g l^{-1} agar fine powder following the protocol of Dupré de Boulois *et al*. (accepted) The initial amount of K in this compartment was 0.14 mg for a concentration of 0.18 mM.

Roots of *M. truncatula* plantlets were subsequently inoculated with spores (± 100) of *Glomus* sp. produced under root-organ culture

(ROC) conditions. Inoculation was performed following the method described by Declerck *et al*. (2003). The bicompartmented Petri plates of the AM-P *in vitro* culture systems were then sealed using Parafilm (Pechiney, Menasha, WI, USA) and wrapped in black plastic bags to maintain roots and *Glomus* sp. in the dark. The AM-P *in vitro* culture systems were subsequently incubated in a controlled environment chamber (20°C, 16 h photoperiod). Daylight lamps provided an average photosynthetic photon flux at the level of the SCs of 225 µmol m^{-2} s^{-1}.

MSR^{Cs} medium was added in the RCs every two weeks to insure adequate plant and fungal growth. The medium (5ml) was added at 35- 40°C in order to reduce the risk of damaging the roots and AM fungi. Addition of new MSR^{Cs} medium in the RCs was stopped 8 weeks after inoculation. The plants therefore received throughout the experiment a total of 0.46 mg of K.

Three weeks after inoculation, AM fungal mycelium started to contact the roots and to develop in the entire volume of the RCs. Eight weeks after inoculation, the HCs were filled with 20 ml of liquid MSR^{Cs} medium, lacking sucrose, vitamins and K. This allowed hyphae to develop in the HCs.

Radio-isotopic labeling

Ten weeks after the beginning of the experiment (i.e. 2 weeks after initiation of hyphal development in the HCs), the liquid medium contained in the HCs was removed with a sterile syringe and immediately replaced by 5 ml of a fresh liquid MSR^{Cs} medium lacking sucrose, vitamins and containing 0, 1 and 10mM K for two concentrations of P, i.e. 30µM and 3000µM. Therefore 6 treatments were considered. K was supplied as KCl and P as $Ca(H_2PO_4)_2$.

Filter-sterilized (Acrodisc® Syringe Filters, PALL Corporation, Ann Arbor, MI, USA) radiocaesium (^{134}Cs, Cs treatment) was added to the liquid medium in the HCs. The concentration of ^{134}Cs was 1531 Bq ml^{-1} in the medium. The source of ^{134}Cs was in form of $CsNO_3$ in water, supplied by the laboratory of nuclear chemistry (CMAT, UCL, Belgium).

A formaldehyde control treatment was included. This control was aimed to determine whether the translocation of ^{134}Cs from the HCs to the RCs was mediated by active processes. Formaldehyde (2% v/v) was introduced two days before labeling (Declerck *et al*., 2003).

For each treatment (no K + 30µM P; no K + 3000µM P; 1mM K + 30µM P; 1mM K + 30000µM P; 10mM K + 30µM P; 10mM K + 3000µM P and control) six replicates were considered.

Harvest and plant – AM fungal analyses

Before labelling, the total extraradical hyphal length and number of spores in the RCs and HCs were estimated. The extraradical hyphae length was determined as detailed in Declerck *et al*. (2003), while spore count followed the methodology described in Declerck *et al*. (2001b). The number of active hyphae (i.e. presenting bi-directional flux of cytoplasm/protoplasm) crossing the partition wall was measured under a dissecting microscope [×40 – Olympus SZ40, Olympus Optical (Europa) Gmbh, Germany].

At the end of the experiment (i.e. 2 weeks after the addition of ^{134}Cs), the shoots of *M. truncatula* were collected by cutting the shoots at the level of the solidified MSR^{Cs} medium contained in the RCs. The liquid medium in the HCs was sampled and the HCs were rinsed twice with 5 ml of distilled water for ^{134}Cs activity measurements. Fungal mycelium developing into the HCs was then collected. In the RCs, roots were removed from the solidified MSR^{Cs} medium and cleaned-free from the remaining gel and extraradical mycelium. The solidified MSR^{Cs} medium containing extraradical mycelium was then collected. Samples were then subjected to ^{134}Cs counting on a Wallac 1480 Gamma Counter (Wallac, Turku, Finland).

Results

Plant and AM fungal growth variables

Plant and AM fungal growth variables are presented in Table 1. At the end of the experiment, *M. truncatula* plants had thin and white roots, as well as green leaves. In both the RCs and HCs, AM fungal mycelium developed profusely as described by Dupré de Boulois *et al*. (2006a). Numerous hyphae crossed the partition wall separating the RCs from the HCs. Statistical analyses showed no difference between the treatments for any of the plant and fungal growth variables. Thus for each treatment, plant and fungal characteristics were equivalent.

Plant and fungal parameters for the control treatment were not significantly different with the others treatments. However, the metabolic activity of the AM fungal mycelium developing in the HC was stopped almost immediately after addition of formaldehyde. This was evidenced by the absence of cytoplasmic/protoplasmic flux in hyphae crossing the partition wall. Trace amounts of ^{134}Cs were detected in the fungal biomass of the HC and in the solidified modified Strullu-Romand medium adapted for Cs studies (MSR^{Cs}) containing also fungal biomass of the RC, but accounted for less than 0.01% of the initial supply of ^{134}Cs in the HC. No ^{134}Cs was detected in the roots or shoots of *M. truncatula*.

Influence of K on Cs transport

In Figures 1a and b are summarized the results obtained on the influence of K on Cs transport at two concentrations of P (30 and 3000µM). For the lowest P concentration, significant differences were obtained for the Cs content in both root and shoot of *M. truncatula* plants, between 0 and 10mM K. No differences in root or shoot Cs content were observed between treatments with 0 and 1mM K and 1mM and 10mM K for this concentration of P. Highest Cs content in the root and shoot (i.e. 39 and 5% of initial Cs supply, respectively) was observed when no K was supplied in the HCs while the lowest was observed when K was supplied at a concentration of 10mM in the HCs (20 and 2% of initial Cs supply, respectively). No difference in Cs content in root and shoot of *M. truncatula* was observed at the highest P concentration whatever the K concentration. Highest root Cs content was measured when no K was added in the HCs while the lowest root Cs content was observed when 10mM K was added to the HCs (i.e. 47% and 32% of initial Cs supply, respectively). Similarly, in the shoots the highest Cs content was measured when no K was added in the HCs, and the lowest when 10mM K was added to the HCs (9 and 6% of initial Cs supply, respectively).

Root/shoot Cs ratios were not significantly affected by increasing K concentrations at the two P concentrations tested. On average, 90 and 85% of Cs was situated in the roots of *M. truncatula* when P was applied at 30µM and 3000µM, respectively.

Influence of P on Cs transport

In Figures 1a and b, the results on the influence of P on Cs transport are presented for each K concentration (0, 1 and 10 mM, respectively). When no K or 1mM K was added in the HCs, no difference was observed in root Cs content at the two concentrations of P tested. However, a significant increase was observed in shoot Cs content at the two concentrations of P applied in the HCs. When K was added at the highest concentration in the HC, a significant increased in both root and shoot Cs contents was observed while P concentrations increased from 30 to 3000µM.

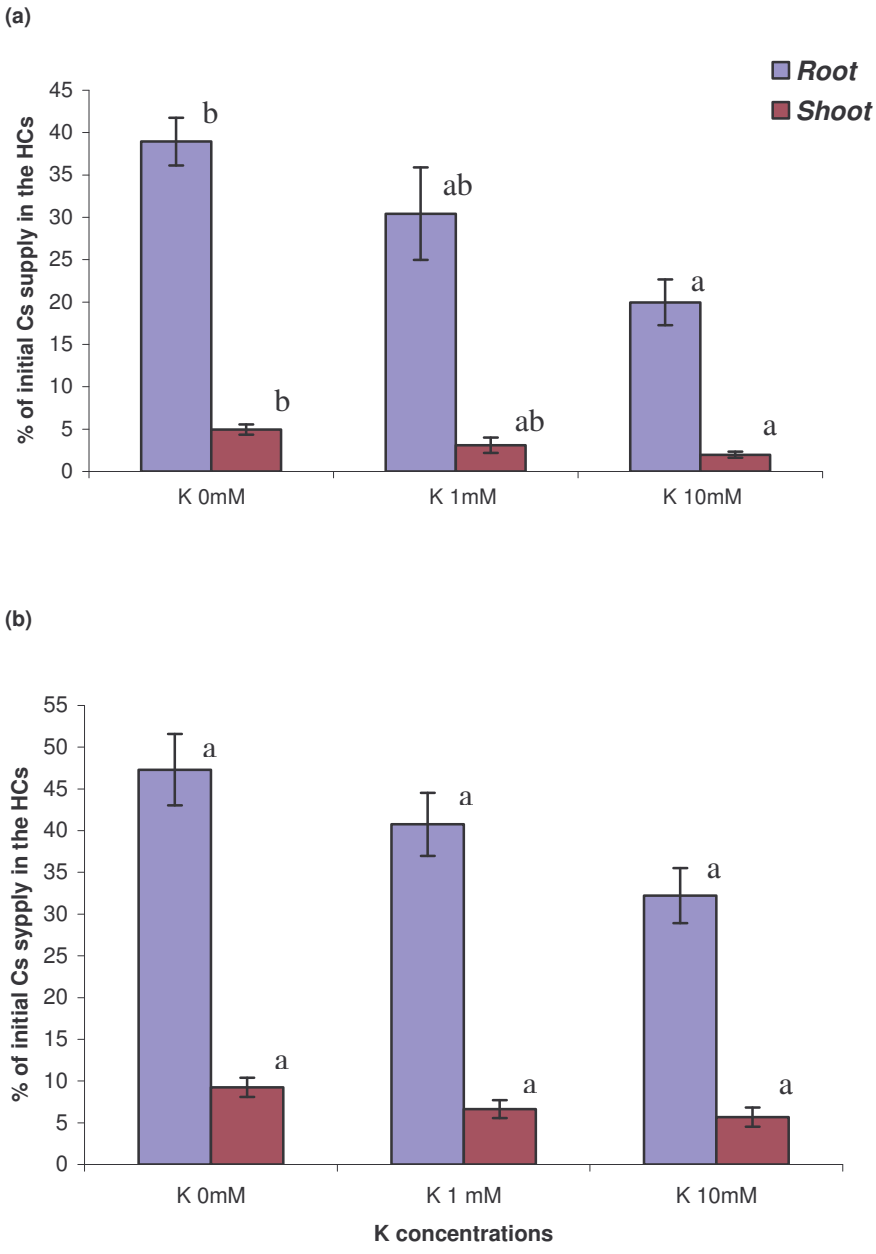
Root/shoot Cs ratios were significantly reduced by increasing P concentrations at the three K concentrations tested. On average, 90% and 85% of Cs was situated in the roots of *M. truncatula* at the lowest and highest P concentration, respectively.

Table 1 Plant and arbuscular mycorrhizal fungal growth variables in the 6 treatments (i.e. P concentrations of 30 or 3000µM for K concentrations of 0, 1 and 10mM), estimated by the shoot fresh weight (SFW), root fresh weight (RFW), hyphal length and number of spores in the root compartment (RC) and hyphal compartment (HC), and the number of active crossing hyphae between the RC and HC .

	P 30µM			**P 3000µM**		
	K 0mM	**K 1 mM**	**K 10 mM**	**K 0mM**	**K 1 mM**	**K 10 mM**
SC						
SFW (mg)	547 ± 60[1]	426 ± 46	575 ± 56	593 ± 83	455 ± 36	642 ± 71
RC						
RWF (mg)	1290 ± 127	1192 ± 426	1255 ± 575	1294 ± 95	1326 ± 455	1359 ± 149
Hyphal length (cm)	471 ± 79	393 ± 79	443 ± 49	556 ± 185	484 ± 82	479 ± 152
Number of spores	366 ± 105	217 ± 59	391 ± 100	445 ± 183	124 ± 15	202 ± 42
HC						
Hyphal length (cm)	1055 ± 226	1017 ± 303	850 ± 148	1108 ± 219	836 ± 194	1161 ± 379
Number of spores	963 ± 280	1038 ± 403	1166 ± 247	1216 ± 345	1041 ± 297	853 ± 154
RC and HC						
Number of active crossing hyphae	29 ± 5	29 ± 5	31 ± 4	33 ± 7	28 ± 5	30 ± 7

[1] Values correspond to the means ± SE of 6 replicates per treatment.

Figure 1 Influence of K (0, 1 and 10mM) on the accumulation of Cs in the root and shoot of *M. trucuncatula* for two P concentrations: (a) 30µM and (b) 3000µM applied in the HCs. Significant differences in % of initial Cs supply in the HCs for root and shoot are identified by a different letter, respectively.



Discussion

In the present study, the transport of Cs was affected by increased concentrations of K at P concentration of 30µM, while at 3000µM P, this effect was not observed. The influence of K, even when significative at the lowest P concentration was not as strong as expected. It can therefore be suspected that K has probably only a small influence on Cs uptake by AM fungi.

Fungal Cs transporters have not been identified yet even if it appeared reasonable to suspect that Cs could be taken up by the transporters of K as it was shown for plants (Rodríguez-Navarro, 2000; White & Broadley, 2000; Zhu & Smolders, 2000). However, a recent study, performed by Baeza *et al*. (2005), tend to confirm the deduction of Yoshida & Muramatsu (1998) who observed that K and Cs do not compete for uptake in fungi. These authors consequently suggested that Cs is probably taken up by a different mechanism than the one for K. The present study, unfortunately do not allow to confirm this supposition as no controls with increasing concentration of stable Cs in the HC were performed. It is also possible that K was rapidly depleted in the HCs. Therefore, as K concentration quickly reached levels for which Cs could be efficiently taken up, the actual effect of K would have been limited. However, if K was transported by AM fungi to their host, plant K nutritional status would have been improved and this could have led to a lower capacity of plants to accumulate Cs (Zhu *et al*., 2000). In the same time, root/shoot Cs ratio should have decreased with increasing plant K nutrition (Buysse *et al*.,

1996; Smolders *et al*., 1996; Maathuis & Sanders, 1997; Pilot *et al*., 2003). However, this was not observed in this study.

A more precise insight of the dynamic of Cs uptake in the presence of increasing concentrations of K would therefore be necessary. The determination and comparison of the effect of K and stable Cs on Cs uptake dynamic should therefore allow to ascertain K influence on Cs uptake, but also to show whether Cs is taken up by K transporters or another mechanism independent of K uptake. Physiological studies on extraradical hyphal membrane to study the effect of these elements on Cs uptake should also be performed and characterization of Cs transporters should be carried out as well.

As previously mentioned, it was hypothesized that P could influence Cs transport by AM fungi and plant Cs accumulation, as K, a chemical analog of Cs, is probably associated to P transport processes in AM fungi but also in plant to maintain osmotic balance (Smith & Read, 1997). In particular, Cs could accompany polyphosphates during P translocation from the extraradical to the intraradical phase of the AM fungi. The results obtained here showed that Cs transport is indeed affected by P as increasing P concentrations in the HCs resulted in a higher accumulation of Cs in *M. truncatula*. However, only significant results on increased root Cs accumulation were obtained when the initial K concentration in the HCs was 10mM. P significantly affected shoot Cs accumulation for any of the three initial K concentration in the HCs. These results therefore suggest that

P can indeed influence Cs transport by AM fungi. Furthermore, Cs transfer from fungal cells to plant cells could be facilitated by P and/or P root to shoot translocation could influence Cs. This is partly confirmed by the root/shoot Cs ratios, as increasing the P supply in the HC resulted in lower Cs root/shoot ratios. However, another reasonable explanation is that P transport by AM fungi could have resulted in a better plant nutritional status that could have resulted for Cs in a plant higher sink-force. Nevertheless, even if differences in Cs accumulation in *M. truncatula* were clearly observed as a result of increasing initial P concentrations in the HC, this study did not allow us to conclude on the precise influence of P on AM Cs transport. To ascertain our hypothesis, it would therefore be needed to measure, dynamically and in parallel, Cs on P depletion in the medium of the HC for various initial P concentrations in this compartment.

In conclusion, the present results did not allow us to determine the role of K and P in Cs transport by AM fungi. However, it appeared that neither K nor P had a strong influence on plant Cs acquisition and accumulation by AM fungi under the present conditions. Further studies should be performed to determine whether Cs is taken up by K transporters and/or other transporters and whether Cs is associated to P in AM transport processes.

DISCUSSION

This work will be submitted to the

Journal of Environmental Radioactivity

(NB: only the part on radiocaesium and arbuscular mycorrhizal fungi is presented here)

Role and influence of mycorrhizal fungi on radionuclide accumulation by plants

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Introduction

Demonstrations of the role of plants and microorganisms in the removal or immobilization of both organic and inorganic soil contaminants underline the potential to employ these organisms as relevant remedial tools. This led to a growing recognition of the necessity to foster research aiming at developing remediation strategies in which their capacity could be used for various contaminants and also in different environmental conditions. The possibility to use plants and micro-organisms in remedial strategies to treat radionuclide contaminated soil was therefore evoked as observations clearly showed that they could participate significantly in

their accumulation and immobilization. In this respect, ecto-mycorrhizal fungi were identified as major contributors of radiocaesium (Cs) immobilization in soils. However, if these fungi could take up, translocate and accumulate significant amount of Cs in their mycelium and carpophores, their role in the transport of this element to their host plants was not determined. In parallel, investigations of the obligate biotrophic arbuscular mycorrhizal (AM) fungi did not allow to determine whether these microorganisms could participate in Cs immobilization in soils or in its accumulation by plants. Here, we aim at reviewing the impact of arbuscular mycorrhizal (AM) fungi on Cs plant acquisition and accumulation. Radioecological significance will be discussed along with the new avenues of research that should be explored to delineate the practical usage of mycorrhizal fungi for phytoremediation strategies of this radionuclide are feasible.

Study methods

Mono-compartmented pot systems

To study and identify the influence of mycorrhizal fungi on radionuclide immobilization and plant accumulation, mono-compartmented pot systems have been widely used. They consist in a pot in which mycorrhizal plants develop in a substrate contaminated with a radionuclide. This approach derives from earlier studies on the

role of mycorrhizal fungi in plant mineral nutrition to compare the growth and mineral nutrient content of mycorrhizal versus non-mycorrhizal plants (Dupré de Boulois *et al*., accepted). Although informative, such system is not adequate to demonstrate *per se* the involvement of mycorrhizal fungi in the uptake of the element studied. Indeed, differences in element content between mycorrhizal and non-mycorrhizal plant could be linked to an indirect effect of the symbiosis on root uptake mechanisms or on the bioavailability and speciation of the element by modification of root exudation. Furthermore, as both extraradical hyphae and roots have access to the element under study, fungal transport is not assessed but rather the combined effect of AM fungi and root uptake. Finally, due to the dilutive effect, the concentration of an element in plant tissues cannot clearly identify the role of mycorrhizal fungi in its acquisition and accumulation.

Compartmented pot systems

To overcome the major limitations of the mono-compartmented pot systems, pot culture systems in which a volume of substrate is inaccessible to the roots were developed (see Schweiger & Jakobsen, 2000 for instance). In these compartmented systems only the fungal extraradical mycelium is allowed to enter a determined volume of substrate in which a radio-tracer isotope of the element of interest is used. These compartmented pot systems have provided a large amount of information on element transport by mycorrhizal fungi (see for

references Marschner, 1995; Schweiger & Jakobsen, 2000; Dupré de Boulois *et al*., accepted) and mycorrhizal mycelium uptake rates (Jakobsen, 1999).

However, if these pot culture systems have led to striking results on element transport and accumulation by mycorrhizal fungi (see for references Marschner, 1995; Schweiger & Jakobsen, 2000), they presented some major drawbacks. Among these are (1) the unpredictable presence of undesirable microorganisms which could influence element bio-availability or transport processes, (2) the difficulty to visualize the development of the two partners of the symbiosis, (3) the difficulty to recover roots and extraradical fungal mycelium, (4) the potential direct uptake of the element studied by the roots due to radiotracer leakage towards the root compartment caused by diffusion or mass-flow, and (5) the complexity to perform physiological and molecular studies on extraradical fungal mycelium and mycorrhizal roots.

In vitro *systems*

Because of the drawbacks of the compartmented pot systems, *in vitro* culture systems became widely used in recent years (Hartley *et al*., 1997; Leyval *et al*., 1997; Fortin *et al*., 2002; Declerck *et al*., 2005) and particularly bi-compartmented systems for AM fungi transport studies (Rufyikiri *et al*., 2005). Studies on tolerance, immobilization and accumulation of pollutants by ECM fungi under *in*

vitro conditions have been performed axenically (see for example Blaudez *et al*., 2000; Colpaert *et al*., 2000). For AM fungi, axenic cultures are not possible due to the obligatory biotrophic nature of these microorganisms. However, monoxenic cultures using root-organs (i.e. an AM fungus associated with excised roots, Declerck *et al*., 2005) are feasible and allowed to perform transport studies where only the fungal partner has access to a labelled compartment (Rufyikiri *et al*., 2005). This spatial separation is achieved by using bi-compartment Petri plates where in one compartment a root and AM fungi are allowed to develop (i.e. the root compartment, RC) and in the other compartment only the extraradical mycelium (i.e. the hyphal compartment, HC) is allowed to grow (Rufyikiri *et al*., 2005). *In vitro* monoxenic cultures generally use *Agrobacterium rhizogenes* Ri T-DNA transformed carrot (*Daucus carota* L.) roots as host (Declerck *et al*., 2005) as they can be easily associated with several species/strains of AM fungi and can be cultured in both solid and liquid media (Cranenbrouck *et al*., 2005). This highly controlled system has been used to study the transport of P and the uptake and translocation of N by AM fungi (Rufyikiri *et al*., 2005). Concurrent uptake and translocation by both the extraradical mycelium and mycorrhizal roots and by non-mycorrhizal roots can also be performed in order to determine the relative contribution of AM fungi and roots in the uptake and translocation of an element (Rufyikiri *et al*., 2005). However, if this system allowed to obtain major results on element transport, its principal limitation was the absence of photosynthetic active tissues (Fortin *et al*., 2002; Dupré de Boulois *et al*., 2006a).

Therefore, following the work of Voets *et al*. (2005), Dupré de Boulois *et al*. (2006a) developed a new system (i.e. Arbuscular Mycorrhizal – Plant (AM-P) *in vitro* culture system) in which a whole autotrophic plant could be associated under *in vitro* conditions to an AM fungus. This system consists of a bi-compartmented Petri plate with a RC and HC and a tube in which the shoot could develop (i.e. the shoot compartment, SC). This system was used to study Cs and P transport by AM fungi while the system of Voets *et al*. (2005) was adapted to perform transport studies (see Dupré de Boulois *et al*., accepted).

Cs transport and immobilization by AM fungi

Preliminary considerations

In ecosystems dominated by herbaceous plants, which are mainly colonized by AM fungi, the capacity of AM fungi to accumulate and transport Cs remained uncertain and even controversial (Dupré de Boulois *et al*., 2006). Indeed, depending on the plant and AM fungal species and on the experimental conditions, a lower (Dighton & Terry, 1996; Berrek & Haselwandter, 2001), similar (Rogers & Williams, 1986; Rosén *et al*., 2005) or even higher (Mc Graw *et al*., 1979; Entry *et al*., 1999; Rosén *et al*., 2005) accumulation of Cs in mycorrhizal plants versus non-mycorrhizal plants was observed. However, as noticed by Joner *et al*. (2004), the experimental

setups used in these studies did not assess fungal transport or immobilization but rather the combined effect of AM fungi and root uptake on plant Cs accumulation. In addition, the participation of AM fungi in the immobilization or transport of Cs could not be evaluated by the sole observation of its accumulation in plants as plant-AM fungus associations and experimental conditions influenced plant growth and root to shoot biomass partition resulting in the well-known "dilution effect" (Timmer & Leyden, 1980; Smith & Read, 1997; Clark & Zeto, 2000). Finally, the influence of soil constituents on K and Cs bio-availability (Delvaux *et al*., 2001), Cs root uptake (White & Broadley, 2000; Zhu & Smolders, 2000; Hampton *et al*. 2005) and Cs plant accumulation (Zhu *et al*., 2000) has not been or scarcely considered, if not even misinterpreted.

Facing the absence of convincing results on the role of AM fungi in the accumulation of Cs in plants, it was necessary to unambiguously determine if the extraradical AM fungal mycelium was able to take up, immobilize and/or translocate Cs towards its host. To do so, the bi-compartment monoxenic culture system using Ri T-DNA transformed carrot roots was used. The classical media used to support the growth of Ri T-DNA transformed carrot roots and AM fungi contained a relatively high concentration of K (Declerck *et al*., 2003). Indeed, solid and liquid MSR media contained respectively 4.38 and 1.65mM K. These concentrations appeared to be inadequate to determine the possible uptake of Cs by AM fungi for two main reasons. The first one is that the nutritional K status of plants is

involved in Cs plant accumulation. Indeed, Zhu *et al*. (2000) observed that K plant starvation results in a stimulation of Cs uptake. Therefore, in plants having a satisfactory nutritional K status, the accumulation of Cs by plants could have been limited. The second reason is that uptake of Cs by fungal K transporters could be limited by K as K and Cs might compete for uptake (Terada *et al*., 1998; Rodríguez-Navarro, 2000). However, Yoshida & Muramatsu (1998) and Baeza *et al.* (2005) suggested that the mechanism of Cs uptake in fungi could be different from that of K. It was nonetheless estimated that reducing K levels in the medium where the extraradical mycelium would take up Cs appeared a minimal precausion (Declerck *et al*., 2003). By imposing low K concentrations in the solid (0.18mM) and no K in the liquid MSR medium, the uptake of Cs was therefore expected to be optimal.

Transport and immobilization of Cs by AM fungi

In a first study conducted under *in vitro* culture conditions, Declerck *et al*. (2003) aimed at demonstrating whether the extraradical mycelium of an AM fungus could take up, translocate and/or immobilize Cs. Results of this study unambiguously showed for the first time that AM fungi were able to perform these processes. Indeed, after two weeks of contact, the extraradical mycelium took up 1.5% of the initial Cs present in the hyphal compartment, and from this, 55% was measured in the roots, demonstrating the ability of this AM fungus to translocate Cs. Cs contained in the extraradical mycelium

(45% of the Cs taken up) was considered as either immobilized intracellularly or as being adsorbed on the surface of the extraradical mycelium. Declerck *et al*. (2003) further suggested that the slow number of hyphae crossing the partition wall in their study could have led to the accumulation of Cs in the extraradical mycelium contained in the labelled compartment due to a bottleneck effect, which could have restricted the translocation of Cs. Indeed, Rufyikiri *et al.* (2003) estimated that a restricted number of hyphae crossing the partition wall could result in an underestimation of the effect of AM fungi on the uptake and translocation of any element. Also, it was suggested by Dupré de Boulois *et al*. (2005b) that the roots in the study of Declerck *et al*. (2003) were old and could have represented an insufficient sink to permit translocation of Cs. To overcome these problems, Dupré de Boulois *et al.* (2005b) slightly modified the bi-compartment Petri plate system used conventionally (1) to allow a higher number of hyphae to cross the partition wall between the RC and HC, and (2) to have actively growing roots throughout the experiment.

The ability, of the AM fungal strain, tested by Declerck *et al*. (2003), to take up and translocate Cs, was confirmed by Dupré de Boulois *et al.* (2005b). In this study the uptake of Cs represented 5.2% of the initial Cs in contact with the extraradical mycelium. These authors estimated that the higher mycorrhizal biomass in the labelled compartment was probably responsible for this higher uptake. Furthermore, they showed that the translocation of Cs by AM fungal mycelium towards the roots represented 81% of the uptake while the

immobilization of Cs in the extraradical represented only 19% of the uptake. This therefore confirmed the suppositions of Declerck *et al.* (2003) and Dupré de Boulois *et al*. (2005b), that low number of crossing hyphae and low root vigour were probably responsible for the high immobilization of Cs in the extraradical mycelium. This was further confirmed by the study of Dupré de Boulois *et al*. (2006a) using autotrophic plants instead of carrot roots.

Following these results obtained under *in vitro* conditions, Joner *et al.* (2004) took the party to perform a new *in vivo* study in order to precise the role of AM fungi in the transport of Cs. To do so, three experiments were conducted. In these experiments, different protocols (i.e. pot systems, substrate, and fertilization) and fungus/host plant combinations were used. Some similarities between these experiments should be considered. The first one was that compartmented pot systems were used where roots and extraradical mycelium could be separated. These systems therefore allowed the distinction between root and AM fungal uptake, and consequently, only fungal hyphae were in contact with Cs. The second was that, in all the substrates used, clay was present. The type of clay and more importantly, Cs sorption capacity of these substrates were however not precised. The third was that K fertilization was made. It could be roughly estimated that K concentration in the soil solution was within the milli-molar range. However, the concentrations of K and also of Cs in the soil solution were not measured by the authors at any time during the courses of these experiments. Therefore, the authors of these study did

not consider the capacity of clay to bind Cs, the effect of K on Cs bio-availability, plant uptake and accumulation and functioning of K/Cs transport processes in both plant and fungi (Sacchi *et al*., 1997; Rodríguez-Navarro, 2000; White & Broadley, 2000; Zhu & Smoders, 2000; Delvaux *et al*., 2001).

Joner *et al*. (2004) concluded that no Cs was transported by the AM fungi tested, and that Cs was not even translocated. It was consequently concluded that AM fungi were not involved in plant Cs acquisition. However, the conclusion of this study could be that the uptake of Cs by AM fungi could be strongly impaired by the sorption capacity of clay, the bio-availability of K in the soil solution and/or plant K nutritional status (Dupré de Boulois *et al*., 2005a).

To address the results obtained by Joner *et al*. (2004), Dupré de Boulois *et al*. (2006a) developed an original *in vitro* culture system, the arbuscular mycorrhizal (AM-P) *in vitro* culture system, in which an AM fungus was associated to an autotrophic plant. With this system, the authors intended to determine, using the same media previously used by Declerck *et al.* (2003) and Dupré de Boulois *et al*. (2005b), but lacking both vitamins and sucrose, whether AM fungi could transfer Cs to their host. Indeed, the absence of photosynthetic tissues renders the monoxenic culture systems using Ri T-DNA transformed carrot roots or excised roots inadequate to show whether an element could be transferred from the intraradical fungal structures of AM symbiosis to the plant tissues. Moreover, the presence of

sucrose in the culture medium, lack of a normal root hormonal balance and incomplete source-sink relationships may have altered plant-fungal symbiotic interactions including bi-directional exchanges (Fortin *et al*., 2002). Using the AM-P *in vitro* culture system, Dupré de Boulois *et al.* (2006a), observed that - after two weeks of contact between Cs and the extraradical mycelium of an AM fungus - Cs was present in the roots and also in the shoots of *M. truncatula* plantlets. This implied that Cs had been taken up by the extraradical mycelium, translocated towards the mycorrhizal roots and transferred to the plant tissues. Uptake by the extraradical mycelium represented 21.0% of the initial supply of ^{134}Cs. From this uptake, 83.6% of the Cs was translocated to *M. truncatula*, the remaining being located in the fungal biomass. Within the plants, 89.8% of the ^{134}Cs was located in the roots, and thus only 10.2% in the shoot.

Potential mechanisms of transport and immobilization of radiocaesium by AM fungi

Mechanisms governing Cs uptake remain speculative. It was suggested that the uptake of Cs is mediated by K transporters as observed for plants (Dupré de Boulois *et al*., 2005b). In fungi, two types of K/Cs transporters coexist (Rodríguez-Navarro, 2000). The high-affinity potassium (HAK) transporters (1 µM K^+ K_m) mediate influx of Cs^+ with low selectivity between K^+ and Cs^+ (Sacchi *et al*., 1997; Rodríguez-Navarro, 2000) and their expression, in plants, is increased markedly at low external K concentrations ($[K^+_{ext}]$) (Santa-

María *et al*., 1997; Hampton *et al*., 2005). The potassium TRK transporters are responsible for the influx of K in fungi at higher [K^+_{ext}] (Rodríguez-Navarro, 2000). In plants, these TRK transporters, which also mediate active K influx, have a millimolar K^+K_m when [K^+_{ext}] is millimolar but their K^+K_m can also decrease continuously down to 0.02 mM under restricted K^+ supply (Haro & Rodríguez-Navarro, 2002). The influx of Cs^+ by these transporters is greatly reduced by increasing [K^+_{ext}] as K^+ is a competitive inhibitor of Cs^+ (Gassmann *et al*., 1996). White & Broadley (2000) therefore estimated that these transporters probably do not mediate Cs^+ influx in plant cells. In fungi, this could likely be the case as well. The occurrence of the HAK transporters should be verified in AM fungi, as they are not found in all fungal species in contrast to the TRK transporters (Durell *et al*., 1999; Rodríguez-Navarro, 2000). These HAK transporters are nervertheless good candidates for Cs uptake at low [K^+_{ext}]. However, Yoshida & Muramatsu (1998) and Baeza *et al.* (2005) suggested that the mechanism of Cs^+ uptake in fungi could be different from that of K^+. Indeed, the uptake of ^{134}Cs by *Pleurotus eryngii* and *Hebeloma cylindrosporum* was not correlated with the content of K in the media and for *P. eryngii*, these authors observed a decrease in the uptake of ^{134}Cs with increasing Cs content in the medium. These observations therefore suggest that if Cs can eventually be taken up by HAK transporters in AM fungi, it cannot be excluded that other transporters might mediate most of the Cs uptake. It should also be noticed that at the concentration of [K^+_{ext}] used by Yoshida & Muramatsu (1998) and Baeza *et al.* (2005), HAK transporters were probably not involved in

K^+ or Cs^+ uptake (Santa-María *et al*., 1997; Hampton *et al*., 2005). This other Cs^+ uptake mechanism in fungi could be compared with what has been observed in plant. Indeed, models on Cs uptake by plants suggested that the majority of Cs enter the root cell plasma-membranes through voltage insentitive cation (VIC) channels (White & Broadley, 2000) that are neither specific to K^+, nor to Cs^+. Exploring the mechanisms of Cs uptake by AM fungi should therefore be pursued carefully and concentrate on the possibility that Cs uptake, at least at higher than 0.25mM [K^+_{ext}], could be predominantly mediated by non-selective cation transporters.

Research efforts to identify Cs transporters in AM fungi are therefore necessary to determine (1) if the HAK transporters are actually present in AM fungi as they are not ubiquous in fungi (Rodiguez-Navarro, 2000) in contrast to the TRK transporters (Durell *et al*., 1999; Haro & Rodríguez-Navarro, 2002), (2) if they can mediate Cs uptake, but also if other transporters might also participate in Cs uptake, and (3) the affinity/permeability of Cs through the HAK and TRK transporters and eventually through these other transporters.

Translocation mechanisms of Cs from the ERM to the intraradical mycelium are yet unknown. Dupré de Boulois *et al*. (2005b) suggested that Cs could be associated with polyphosphates. Indeed, after the uptake of Pi by the extraradical mycelium via an active transport mechanism, a fraction of it (the other being directly used by the fungus for its own metabolism) is transferred into

vacuoles and condensed into polyphosphate, which are strong negatively charge polyanions structures (Viereck *et al*., 2004). In order to maintain osmosis, it has been suggested that cations, such as K and Mg are associated with these structures to balance these negative charges (Orlovich & Ashford, 1993; Cole *et al*., 1998; Jentschke *et al*., 2001; Ryan *et al*., 2003). Once in these vacuoles, the translocation of polyphosphates and associated cations is mediated by the motility of these vacuoles along chains referred as the motile tubular vacuolar system. This system therefore ensures the translocation of polyphosphates from the site of uptake to the symbiotic fungal-plant exchange interface (Ezawa *et al*., 2002). Therefore, polyphosphates could act as a strong and motile Cs sink within fungal hyphae, in case Cs can also be associated to these structures. However, the immobilization of Cs by the polyphosphates in this vacuolar system could only be considered as a temporal or transitory mechanism. Indeed, when the polyphosphates reach the intraradical mycelium and further at the sites of P transfer, they are hydrolysed to release Pi (Ezawa *et al*., 2002; Viereck *et al*., 2004). In this process, K is probably released within fungal intraradical structures. However, it seems that this hydrolytic enzyme activity is rather slow and therefore results in an accumulation of polyphosphates in intraradical mycelium (Ezawa *et al*., 2002; Ryan *et al*., 2003). Due to this accumulation, it can be suspected that K could also accumulate in the intraradical fungal hyphae before being transferred and/or immobilized. In intraradical hyphae, Ryan *et al*. (2003) indeed clearly showed that K and P accumulations were correlated. It has also to be

noticed that K can accumulate in vesicles with other elements such as U, Mn, Cu, Fe and Ni (Kaldorf *et al*., 1999; Weiersbye *et al*., 1999). Results on Cs are yet to be obtained, but Cs can be expected to behave in a similar way as K in its translocation from extra- to intra-radical fungal structures.

The role of K/Cs as potential balancing cations of polyphosphates during translocation raised another question. Indeed, if translocation of K/Cs is dependent of the one of P, it means that dependency of plant hosts to acquire P from AM fungi, but also the capacity of AM fungi to transport P will directly influence the transport of K/Cs. This is of particular interest in the study of Cs transport if it is considered that most of the P present in the HC, in the experiments previously cited, was taken up and translocated within a few days after labelling (Maldonado-Mendoza *et al*., 2001; Nielsen *et al*., 2002). Indeed, it can be supposed that the translocation of Cs would be highly limited thereafter. This hypothesis was tested in a recent experiment, in which various concentration of Pi were applied in the HC. However, due to limits in the experimental design, it was difficult to determine whether P transport could have actually influenced the one of Cs (unpublished data).

The transfer of Cs from AM fungi to plants was demonstrated for the first time by Dupré de Boulois *et al*. (2006a). However, the mechanism remains unknown even if it can be assumed that the bio-availability of K and K nutritional status of AM fungi and host plant

are probably important as both expression and activity of K/Cs transporters could be affected. Indeed, if the efflux of Cs from intraradical fungal mycelium was mediated by KOR channels (Rodríguez-Navarro, 2000; Corratgé *et al*., 2006) or simple gradient diffusion, the concentration of K^+ in the interfacial apoplast would influence Cs^+ efflux (Ketchum *et al*., 1995). Also, as K^+ is vital for all living organisms, it could be suspected that in case of low $[K^+_{ext}]$, internal K^+ concentrations $[K^+_{int}]$ within AM fungal hyphae might limit K/Cs efflux through KOR channel by down-regulation of their expression (Pilot *et al*., 2003) or by limiting their probably of openness (Maathuis & Sanders, 1997) similarly to stellar KOR (SKOR) channels of plants. The transporters involved in the influx of K/Cs to the host cells would also be affected by K concentration in the interfacial apoplast and the concentration of K within the host cells themselves. It is considered that the influx of Cs^+ in plant cells involved either high-affinity or low-affinity transporters depending on root cell $[K^+_{ext}]$ (see for review White & Broadley, 2000, Zhu & Smolders, 2000). The fact that high-affinity (i.e. HAK) transporters have a low discrimination towards K^+ and Cs^+, influx of Cs^+ can be mediated in root cells at low $[K^+_{ext}]$ (Schachtman & Schroeder, 1994). However, White & Broadley (2000) estimated that VIC channels were probably responsible for most of Cs^+ influx in roots cells at low $[K^+_{ext}]$. When $[K^+_{ext}]$ increase (typically starting at 0.25-0.5 mM), the expression of HAK transporters is down-regulated and Cs^+ influx by VIC channels is inhibited. The influx of Cs^+ could still be operated by low affinity KIR channels (Rodríguez-Navarro, 2000). However, these

channels have a strong discrimination between K^+ and Cs^+ to the advantage of K^+ (Schachtman *et al*., 1992), and therefore do not participate in Cs^+ uptake (White & Broadley, 2000, Zhu & Smolders, 2000). As a consequence apoplastic K^+ concentration can influence the possibility of Cs^+ to enter roots cells even after it has been successfully transmitted by AM fungi.

To resume the possible mechanisms of Cs^+ transfer from AM fungi to root cells, it appears that (i) in limited K^+ bio-availability, efflux of Cs^+ could be minimal from intraradical hyphae and influx into root cells would be efficiently mediated by high-affinity transporters, (ii) in high K^+ bio-availability, efflux of Cs^+ from intraradical hyphae would be efficient, but its influx into root cells would be limited.

Immobilization of Cs by the AM fungal mycelium appeared to be low (Dupré de Boulois *et al*., 2005b; 2006a). However, it should be noticed that the density of mycelium in these studies was limited and therefore, in natural ecosystems where mycelium density can reach several meters per gram of soil, mycelium could immobilize significant amount of Cs. Similarly to that of U, the binding capacity of mycorrhizal fungi may, at least in part be accounted for the presence of chitin on the fungal cell-wall, which is an important constituent of the mycelium of filamentous fungi (Salton, 1960) such as mycorrhizal fungi (Galli *et al*., 1994). Also, glomalin, a glycoprotein, which is firmly incorporated into the wall of AM hyphae

(Driver *et al*., 2005), could participate in Cs sequestration as it has been observed for other cations (González-Chávez *et al*., 2004). Indeed, these proteins contain free amino, hydroxyl, carboxyl and other groups that can be excellent binding sites for cations (González-Chávez *et al*., 2004). It should also be mentioned that Cs could accumulate in the intraradical mycelium of AM fungi as previously mentioned. However, further studies need to be conducted to confirm this hypothesis.

Comparison of the contribution of extraradical mycelium, mycorrhizal roots and non-mycorrhizal roots to the uptake and translocation of Cs

Dupré de Boulois *et al*. (2005b) compared and quantified the respective contribution of the extraradical hyphal network, roots and, both in combination, on Cs uptake and translocation. In their study, they observed that uptake of Cs by the mycorrhizal roots and non-mycorrhizal roots was much higher than when only AM hyphae were in contact with the medium containing Cs. However, Cs taken up in these roots, mycorrhizal or not, remained mostly in the labelled compartments. Only 19.2 and 16.5% of the Cs taken up was actually translocated towards the roots contained in the non-labelled compartment, respectively. This led to the observation that the amount of Cs translocated by hyphae, mycorrhizal roots or non-mycorrhizal roots was identical between the different treatments. Therefore, this implied that Cs was immobilized within the roots and that the translocation of Cs by fungal hyphae was more efficient than that of

the roots, mycorrhizal or not.

In this experiment, the reason for the apparent immobilization of Cs in the roots was poorly understood. As suggested by Dupré de Boulois *et al*. (2005b), it could be due to the lack of an aerial part that would have represented a strong sink for Cs. The authors of this study also proposed that Cs remained in the root cells and were not translocated due to K starvation. Indeed, the transport of Cs towards the xylem could have been impaired to prevent leakage of K from the root cells as previously discussed.

Trans-root Cs movement

Effect of K on trans-root Cs movement

Availability of K^+ in soil affects the accumulation of Cs in plants and its translocation from roots to shoot. When the concentration of K^+ is low (<0.25mM), the uptake of K/Cs is mediated by HAK transporters and VIC channels (White & Broadley, 2000; Zhu & Smolders, 2000). In these conditions, the efflux of K/Cs towards the xylem is limited to prevent K to leave root cells. The KOR channels, which mediate the majority of K^+ and Cs^+ efflux from the root cells (White & Broadley, 2000; Zhu & Smolders, 2000), has indeed a probability of openness most likely close to zero when $[K^+_{int}]$ is low (Maathuis & Sanders, 1997). Moreover, Pilot *et al*. (2003)

observed that in K-starved plants, the expression of genes encoding for SKOR channels in *Arabidopsis thaliana* was reduced. Therefore, the limited efflux of Cs to the xylem could be responsible for the local accumulation of Cs in the roots (Zhu *et al*., 1999). This corroborates the results of Buysse *et al*. (1996) and Smolders *et al.* (1996) who observed that root to shoot Cs concentration ratio increases with decreasing K supply.

When $[K^+_{ext}]$ reaches values of 0.25mM the uptake of Cs is impaired by the down-regulation of HAK transporters (Santa-María *et al*., 1997; Kim *et al*., 1998) and K^+ inhibition of the VIC channels. In addition, Cs^+ influx cannot be mediated by KIR channels due to their strong discrimination between K and Cs (White & Broadley, 2000; Zhu & Smolders, 2000). In this situation, Cs^+ uptake is highly limited. However, as plants have potentially a satisfactory K nutritional status, Cs^+ loading in the xylem is probably optimal. This signifies that (1) plant Cs accumulation would be minimal, but (2) that Cs translocation to the shoot would not be impaired, and therefore that Cs can accumulate in the shoot.

To explain further the relation between K limitation or K deficiency and root to shoot translocation of K, a possible mechanism should be discussed here. Indeed, under these situations, it was noticed that the level of abscisic acid (ABA) in roots increased and could significantly affect Cs loading into the xylem (Peuke *et al*., 2002; Battal *et al.*, 2003). ABA is recognised to play regulatory roles

in physiological processes in all higher as well as in lower plants (Zeevaart & Creelman, 1988; Davies & Jones, 1991). Among these processes, ABA affects plant architecture, including root growth and morphology (i.e. control of elongation and lateral root development), root-to-shoot biomass ratios and geotropism. This phytohormone is also active in water uptake regulation and, more importantly, in ion transport regulation. Early observations showed that ABA could significantly reduce root to shoot K translocation (Roberts, 1998; Roberts & Snowman, 2000). Thereafter, it has been demonstrated that ABA can reduce the expression of genes encoding KOR channels (Gaymard *et al*., 1998; Ache *et al*., 2000, Becker *et al*., 2003; Pilot *et al*., 2003). In roots, the down-regulation of the genes encoding these channels (Gaymard *et al*., 1998; Pilot *et al*., 2003) led to a decrease in the number of KOR channels and consequently K translocation to the shoot was lowered. Moreover, it is possible that ABA could also influence the activity of the KOR channels by modifying the pH of the root cell cytoplasm. Indeed, ABA was shown to reduce root cell cytoplasmic pH (Beffagna *et al*., 1997), which could result in a strong inhibition of KOR channels as observed by Lacombe *et al*. (2000). It is also to be noticed that the expression of HAK transporters and KIR channels appeared insensitive to increasing ABA root levels (Roberts *et al*., 1998; Pilot *et al*, 2003), but not data exist on the effect of ABA on VIC channels to our knowledge.

Finally, the regulation of KOR channel activity can also be mediated by cytosolic free Ca^{2+} ($[Ca^{2+}]_{cyt}$). Indeed, Wegner and De

Boer (1997) observed the control of SKOR channels activity by $[Ca^{2+}]_{cyt}$ and Roberts and Snowman (2000) showed a similar action of ABA and $[Ca^{2+}]_{cyt}$ on KOR channel activity. Finally, Gilliham & Tester (2005) proposed that the reduction of anions loading into the xylem could also result from an increased ABA root concentration, which would stimulate the entry of Ca^{2+} into the cytosol of stellar cells, and thus down-regulates xylem-parenchyma quickly-activating anion conductance (X-QUAC) activity. However, if Cl^- and NO_3^- fluxes were observed through these channels, Pi loading into the xylem might be mediated by another class of channels that is insensible to $[Ca^{2+}]_{cyt}$ (see Köhler & Raschke, 2000 and Gilliham & Tester, 2005). Nevertheless, the possible "coupling" of K^+ with anion loading, and in particular Pi, into the xylem, dependant of an ABA-$[Ca^{2+}]_{cyt}$ signalling pathway, would nevertheless bear sense as transport of these elements are closely linked for reasons of overall charge neutrality (Lin, 1981; Kochian *et al*., 1985; Roberts, 2006).

Effect of AM fungi on trans-root Cs movement

Dupré de Boulois *et al*. (2005b) suggested that AM fungi might play a role in the accumulation of Cs in roots and trans-root Cs movement. In other words, it was suspected that AM fungi might negatively affect Cs loading into the xylem by influencing the expression or activity of KOR channels.

As previously mentioned, the regulation of several genes encoding for root cell plasma-membrane transporters is affected by AM colonization (Rausch *et al*., 2001 and reviews by Burleigh & Bechmann, 2002 and Ferrol *et al*., 2002). Indeed, as AM colonization appears and spreads, interactions between the partners of the symbiosis lead to various modifications of the two partners. For instance, Bago *et al*. (2000), Burleigh & Bechmann (2002) and Bücking & Shachar-Hill (2005) suggested a tight regulation between plant nutritional status, photosynthesis and bi-directional carbon and nutrient exchange at the symbiotic interface. Such regulations involve signals from both partners and their environments. Phytohormones, or plant growth regulators, are probably involved in this dialog (Smith & Gianinazzi-Pearson, 1988; Schwab *et al*., 1991; Beyrle, 1995; Barker & Tagu, 2000; Ludwig-Müller, 2000; Harrison, 2005). Following root colonization, hormone levels and balances can be affected (Beyrle, 1995). One of the hormones to be affected is ABA, which present increased levels in mycorrhizal roots (Danneberg *et al*., 1993; Bothe *et al*., 1994; Ludwig-Muller, 2000). Therefore, AM fungi by increasing ABA root levels could affect the regulation and activity of SKOR channels and thus reduce Cs root to shoot translocation. Investigations ought therefore to be directed on the role of ABA on Cs root to shoot translocation and on the potential influence of AM fungi on this process.

Conclusion

The dynamic of radionuclides in soils depends not only on their physico-chemical interactions with inorganic and organic soil constituents but also on biological interaction associated, to a large extent, with the microbial activity of the soil–plant system. Only recently the importance of microorganism–radionuclide interaction in relation to environmental health and remediation strategies has been considered. This review presented the most recent results obtained on the participation of ubiquous soil fungi on plant Cs accumulation.

It was rapidly shown that ECM fungi could accumulate significant amount of radiocaesium. Therefore, after the disaster of Chernobyl, harvest and consumption of ECM fruiting bodies in contaminated sites was restricted, if not outright forbidden. For researchers it also became clear that ECM fungi could play a role in the immobilization of Cs in soils and thus could participate in their stabilization. However, if many studies investigated the potential of ECM fungi to accumulate Cs, they only scarcely considered the possibility that these fungi could also enhance Cs accumulation by plants.

The capacity of AM fungi to accumulate or transport Cs to their hosts was also investigated. However, the first studies examining the role of AM fungi offered rather insufficient results. Indeed, the capacity of mycorrhizal fungi to transport Cs was not properly

considered as these studies did not allow to differentiate between fungal and root Cs uptake. In addition, due to the difficulty to recover AM fungal mycelium from soil and to distinguish it from the one of other fungi, the capacity of AM fungi to accumulate Cs was not possible to determine.

With the development of compartmented systems, and in particular of *in vitro* compartmented systems, it became possible to determine whether AM fungi could transport and accumulate Cs. Using these systems, we unanbiguously demonstrated that AM fungi could transport Cs to their host, and that accumulation of Cs by extraradical mycelium of AM fungi was rather limited. However, accumulation of Cs in the intraradical mycelium is likely. We also demonstrated that the role of AM fungi was not only restricted to these phenomena as AM fungi appeared to have a strong effect on Cs distribution within their host. Indeed, it seemed that AM fungi could limit the translocation of Cs to their host shoots. Further studies would, of course, be necessary to ascertain that AM fungi can restrict trans-root Cs movement. In this respect, a European project, MYCOREMED ("Role of arbuscular mycorrhizal fungi on the accumulation of radiocaesium by plants"), will aim at determining the role of AM fungi on the expression of transporters involved in Cs loading into the xylem.

However, if the results obtained so far have shown the capacity of AM fungi to influence the acquisition and accumulation of Cs by

plants by immobilizing, transporting and affecting root to shoot translocation of Cs, their ability to take part in phytoremediation strategies remains questionable and would need further investigations. In particular, other fungal species and plant species should be tested. Indeed, variations in Cs soil-to-plant transfer have been reported among and within plant species (Coughtrey and Thorne, 1983, Desmet *et al*., 1990, Frissel *et al*., 2002 and Payne *et al*., 2004) supporting the influence of plant genotype in radiocaesium uptake (Buysse *et al*., 1996). Furthermore, Broadley *et al*. (1999) demonstrated that variations of Cs accumulation in shoot could be identified at different taxonomic levels. Such variations in the capacity of ectomycorrhizal fungi to take up and accumulate Cs has already been shown (Nikolova *et al*., 1997; Steiner *et al*., 2002) and are probably also existing among AM fungal species or groups. The contribution of AM fungi and root uptake in the accumulation of Cs in plants should be further compared and this under various conditions, including increasing K and P levels. Attention should also be focused on the validation of the findings obtained *in vitro* to *in situ* conditions.

CONCLUSIONS
&
PERSPECTIVES

Conclusions

Radiocaesium (Cs) pollution of agricultural, semi-natural and natural areas is a world-wide problem that arose from human activities such as industries, weapon testing and nuclear accidents. The chemical similarity of Cs and potassium (K) constitutes the major threat to the contamination of the aboveground vegetation as root uptake mechanisms appear closely related for these elements. As a consequence, the principal route for Cs entry into the food chain is *via* the soil-to-plant pathway.

Rhizospheric processes involving soil microorganisms are known to influence root uptake of Cs. Among them, the arbuscular mycorrhizal (AM) fungal symbionts have been suspected to play a key role.

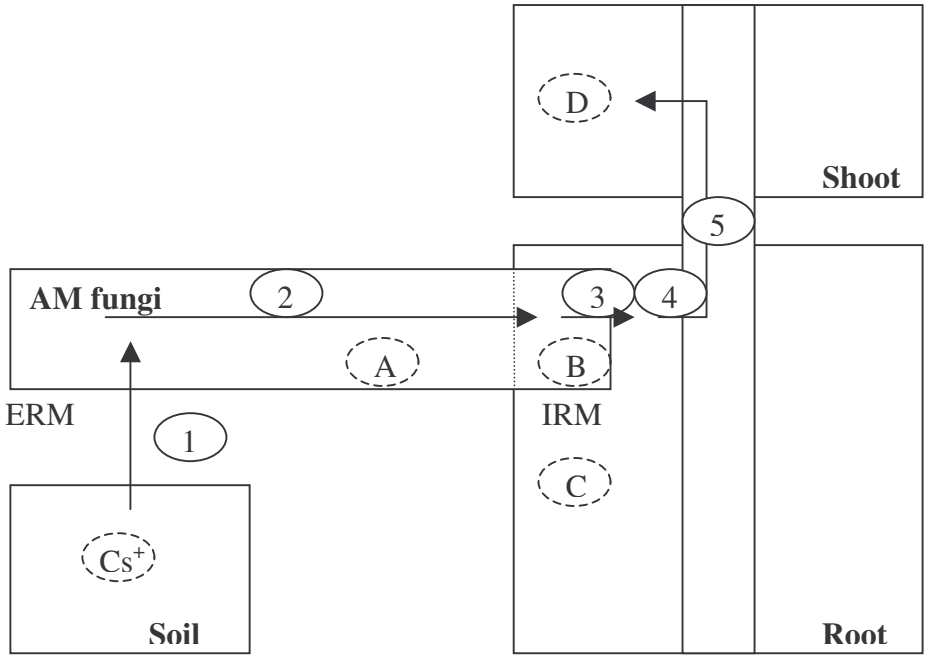
However, the role of AM fungi on the acquisition of Cs by plants remained unknown at the beginning of this work. The objective of this study was therefore to determine whether AM fungi can accumulate Cs or transport Cs to their hosts.

With the development of different and innovative compartmented *in vitro* culture systems, using root-organ cultures (ROC) or autotrophic plants (arbuscular mycorrhizal – plant (AM-P) *in vitro* culture system), it became possible to precisely study the capacity of AM fungi to transport and accumulate Cs. In these

compartmented systems, the extraradical hyphae of AM fungi were physically separated from the roots, so that AM fungal contribution to the acquisition of Cs their hosts could be determined, as well as Cs accumulation in the extraradical mycelium (ERM) of these fungi. The extraradical hyphae developed in a hyphal compartment (HC), which was labelled with Cs, while mycorrhizal roots grew in a root compartment (RC).

The majors results obtained during the course of this work are presented in Figure 1. Using these *in vitro* culture systems, uptake, translocation and transfer of Cs from AM fungi to their host roots have been evidenced while accumulation of Cs in the ERM was low (Table 1).

Fig. 1 Schematic representation of the principal results obtained on how arbuscular mycorrhizal fungi could influence Cs acquisition and accumulation by plants.



1 Uptake of Cs by the extraradical hyphae of AM fungi, see Chapter I.

2 Translocation of Cs from the extraradical mycelium (ERM) to the intraradical mycelium (IRM), see Chapter I.

3 Transfer of Cs from the IRM to the root cells, see Chapter III & IV.

4 Trans-root Cs movement and in particular Cs loading into the xylem could be affected by AM fungi, see Chapter II.

5 Root to shoot Cs translocation following transport (1, 2 & 3) of Cs by AM fungi, see Chapter III & IV.

A Low accumulation of Cs in the ERM following uptake (1) of Cs by AM fungi, see Chapter I & II.

B Potential accumulation of Cs in the IRM following uptake (1) and translocation (2) of Cs by AM fungi, see Chapter II, III & IV.

C Potential accumulation of Cs in roots following transport (1, 2 & 3) of Cs by AM fungi and/or Cs root uptake (4), see Chapter II & III.

D Accumulation of Cs in the shoot following root to shoot Cs translocation (5), see Chapter III, IV.

It should be noticed that the uptake by AM fungi significantly increased during the course of this research project - from 1.5 to 45.9% of the initial supply of Cs in the HC. This marked increased could mainly be attributed to the successive improvements of the systems used to study Cs transport by AM fungi. They indeed allowed to obtain higher fungal biomass in contact with Cs (i.e. about 10-fold increase). The replacement of root-organs by autotrophic plants as host of the AM fungus tested probably had also a very important effect on the transport of Cs by AM fungi due to the presence of an additional sink, i.e. the shoot.

In addition, even though AM fungi participated in plant Cs acquisition, it was shown that Cs translocation from roots to shoot was low. Indeed, only about 10% of the Cs translocated by AM fungi was measured in the shoot. This could be due to the accumulation of Cs in intraradical structures of the AM fungi and/or the influence of AM fungi on trans-root Cs movement, thus limiting root to shoot Cs translocation. It also appeared that K could reduce Cs transport by AM fungi, while P could increase it. However, the effect of these elements on Cs transport by AM fungi was limited. It could therefore be concluded that Cs could be taken up by AM fungi by other transporters than the ones of K, and that the transport of Cs could not be increased by the one of P even so Cs could play the role of balancing cation of P during this process.

Table 1. Overview of the results obtained on the transport of radiocaesium (Cs) by arbuscular mycorrhizal fungi to their host and on Cs accumulation by the extraradical mycelium (ERM) of these plant symbionts.

Results presented in:	System used	% of initial Cs supply				
		Total uptake	ERM in HC	ERM and medium in the RC	Roots	Shoot
Chapter I	ROC	1.5	0.5	0.2	0.8	-
Chapter II	ROC	5.2	0.4	0.60	4.2	-
Chapter III	AM-P	21.0	0.1	3.2	15.9	1.8
Chapter IV	AM-P	45.9	0.2	1.8	38.9	5.0

These results, therefore clearly demostrated that AM fungi could participate in plant Cs accumulation. However, the importance of this participation, as compare with Cs root uptake, remains to be precised. In addition, the suspected role of AM fungi in the restriction of Cs root to shoot translocation needs to be unambiguously demonstrated.

Perspectives

Even though the results of this work showed that AM fungi can participate in the transport of Cs to their hosts, AM fungal participation in plant Cs accumulation should be further compared with root Cs uptake. This comparison, should also include various AM fungal species associated to several host plant species as AM fungal transport and root uptake capacity can greatly differ. Further efforts are also necessary to determine if AM fungi can immobilize Cs in their intraradical mycelium and if they can affect trans-root Cs movements. In this respect, investigations on the mechanisms involved in the transport of Cs by AM fungi, the effect of AM fungi on trans-root movements and thus on root to shoot translocation are required. In particular, the role of abscisic acid (ABA), should be studied as it might affect Cs transfer from AM fungal cells to roots cells, but also Cs loading into the xylem. Furthermore, these studies should be implemented by evaluations of the radioecological significance of the role of AM fungi on Cs stabilization. In this respect, early assessments of the technological feasibility and advantages of AM fungal potential for sustainable remediation strategies should be carried out.

Based on these considerations, future objectives are to (1) determine the mechanisms of Cs transport by AM fungi and if Cs transport is affected by K and P, in particular, (2) identify and study the expression of K/Cs transporters in mycorrhizal and non-

mycorrhizal roots, (3) investigate ABA regulatory pathways involved in K/Cs trans-root movements, and (4) examine the radioecological significance of the influence of AM fungi on Cs plant accumulation by testing various environmental conditions and AM fungus-host plant combinaisons.

Finally, it should be pointed out that the first three of these objectives are currently answered using the HAM-P and AM-P *in vitro* culture sytems that have been developed during this work. In addition, in the laboratory of Prof. S. Declerck, these systems are currently used to study ^{86}Rb, ^{33}P and ^{13}C transport, effects of fungicides, AM symbiosis-pathogen interactions, and also to establish AM fungal strains and species that are recalcitrant to *in vitro* culture on root-organs.

REFERENCES

Ache, P., Becker, D., Ivashikina, N., Dietrich, P., Roelfsema, M.R.G., Hedrich, R., 2000. GORK, a delayed outward rectifier expressed in guard cells of *Arabidopsis thaliana*, is a K^+-selective, K^+-sensing ion channel. FEBS Lett. 486: 93-98.

Alexander, T., Toth, R., Meier, R., Weber, H.C., 1989. Dynamics of arbuscule development and degeneration in onion, bean, and tomato with reference to vesicular-arbuscular mycorrhizae in grasses. Can. J. Bot. 67: 2505-2513.

Ames, R.N., Reid, C.P.P., Porter, L.K., Cambardella, C., 1983. Hyphal uptake and transport of nitrogen from two ^{15}N-labelled sources by *Glomus mosseae*, a vesicular-arbuscular mycorrhizal fungus. New Phytol. 95: 381-396.

Amundsen, I., Gulde, G., Strand, P., 1996. Accumulation and long term behaviour of radiocaesium in Norwegian fungi. Sci. Total Environ. 184: 163-171.

Andrade, G., Mihara, K.L., Linderman, R.G., Bethlenfalvay, G.J., 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular mycorrhizal fungi. Plant Soil 192: 71-79.

Ashford, A.E., Allaway, W.G., 2002. The role of the motile tubular vacuole system in mycorrhizal fungi. Plant Soil 244: 177-187.

Avery, S.V., 1996. Fate of caesium in the environment: Distribution between the abiotic and biotic components of aquatic and terrestrial ecosystems. J. Environ. Radioactiv. 30(2): 139-171.

Baeza, A., Guillen, J., Hernandez, S., Salas, A., Bernedo, M., Manjon, J.L., Moreno, G., 2005. Influence of the nutritional mechanism of fungi (mycorrhize/saprophyte) on the uptake of radionuclides by mycelium. Radiochem. Acta 93 (4): 233-238.

Bago, B., 2000. Putative sites for nutrient uptake in arbuscular mycorrhizal fungi. Plant Soil 226: 263-274.

Bago, B., Cano, C., 2005. Breaking myths on arbuscular mycorrhizas in vitro biology, in: Declerck, S., Strullu, D.G., Fortin, J.A. (Eds.), In vitro culture of mycorrhizas. Springer-Verlag, Heidelberg, pp. 111-138.

Bago, B., Azcon-Aguilar, C., Piché, Y., 1998. Architecture and developmental dynamics of the external mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* grown under monoxenic conditions. Mycologia 90: 52-62.

Bago, B., Pfeffer, P.E., Shachar-Hill, Y., 2000. Carbon metabolism and transport in arbuscular mycorrhizas. Plant Physiol. 124: 949-957.

Bago, B., Pfeffer, P.E., Zipfel, W., Lammers, P., Shachar-Hill, Y., 2002. Tracking metabolism and imaging transport in arbuscular mycorrhizal fungi. Metabolism and transport in AM fungi. Plant Soil 244: 189-197.

Bakken, L.R., Olsen, R.A., 1990. Accumulation of radiocaesium in fungi. Can. J. Microbiol. 36: 704-710.

Barber, S.A., 1962. A diffusion and mass flow concept of soil nutrient availability. Soil Sci. 93: 39-49.

Barkay, T., Schaefe, J., 2001. Metal and radionuclide bioremediation: issues, considerations and potentials. Curr. Opin. Microbiol. 4: 318-323.

Barker, S.J., Tagu, D., 2000. The roles of auxins and cytokinins in mycorrhizal symbioses. J. Plant Growth Regul. 19: 144-154.

Barnett, C.L., Beresford, N.A., Self, P.L., Howard, B.J., Frankland, J.C., Fulker, M.J., Dodd, B.A. Marriott, J.V.R., 1999. Radiocaesium activity concentrations in the fruitbodies of macrofungi in Great Britain and an assessment of dietary intake habits. Sci. Total Environ. 231: 67-83.

Battal, P., Turker, M., Tileklioglu, B., 2003. Effects of different mineral nutrients on abscisic acid in maize (*Zea mays*). Ann. Bot. Fenn. 40(5): 301-308.

Bécard, G., Fortin, J.A., 1988. Early events of vesicular-arbuscular mycorrhiza formation on Ri T-DNA transformed roots. New Phytol. 108: 211-218.

Bécard, G., Kosuta, S., Tamasloukht, M., Sejalon-Delmas, N., Roux, C., 2004. Partner communication in the arbuscular mycorrhizal interaction. Can. J. Bot. 82(8): 1186-1197.

Becker, D., Hoth, S., Ache, P., Wenkel, S., Roelfsema, M.R., Meyerhoff, O., Hartung, W., Hedrich, R., 2003. Regulation of the ABA-sensitive Arabidopsis potassium channel gene GORK in response to water stress. FEBS Lett. 554: 119-126.

Beffagna, N., Romani, G., Meraviglia, G., Pallini, S., 1997. Effects of abscisic acid and cytoplasmic pH on potassium and chloride efflux in *Arabidopsis thaliana* seedlings. Plant Cell Physiol. 38: 503-510.

Bell, J.N.B., Minski, M.J., Grogan, H.A., 1988. Plant uptake of radionuclides. Soil Use Manage. 4: 76-84.

Bellion, M., Courbot, M., Jacob, C., Blaudez, D., Chalot, M., 2006. Extracellular and cellular mechanisms sustaining metal tolerance in ectomycorrhizal fungi. FEMS Microl. Lett. 254 (2): 173-181.

Berreck, M., Haselwandter, K., 2001. Effect of the arbuscular mycorrhizal symbiosis upon uptake of cesium and other cations by plants. Mycorrhiza 10: 275-280.

Berthelsen, B., Olsen, R.A., Steinnes, E., 1995. Ectomycorrhizal heavy metal accumulation as a contributing factor to heavy metal levels in organic surface soils. Sci. Tot. Environ. 170: 141-149.

Beyrle, H., 1995. The Role of phytohormones in the function and biology of mycorrhizas, in: Varma, A., Hock, B. (Eds.), Mycorrhiza structure, function, molecular biology and biotechnology. Springer-Verlag, Berlin, pp. 365-390.

Blaudez, D., Botton, B., Chalot, M., 2000. Cadmium uptake and subcellular compartmentation in the ectomycorrhizal fungus *Paxillus involutus*. Microbiology 146: 1109-1117.

Blee, K.A., Anderson, A.J., 1998. Regulation of arbuscule formation by carbon in the plant. Plant J. 16: 523-530.

Blee, K.A., Anderson, A.J., 2002. Transcripts for genes encoding soluble acid invertase and sucrose synthase accumulate in root tip and cortical cells containing mycorrhizal arbuscules. Plant Mol. Biol. 50(2): 197-211.

Bostock, R.M., 2005. Signal crosstalk and induced resistance: Straddling the line between cost and benefit. Annu. Rev. Phytopathol. 43: 545-580.

Bothe, H., Klingner, A., Kaldorf, M., Schmitz, O., Esch, H., Hundeshagen, B., Kernebeck, H., 1994. Biochemical approaches to the study of plant-fungal interactions in arbuscular mycorrhiza. Experientia 50: 919-925.

Bowen, H.J.M., 1979. Environmental Chemistry of the Elements. Academic Press, London, UK.

Bradley, R., Burt, A.J., Read, D.J., 1982. The biology of mycorrhiza in the Ericaceae. VIII. The role of mycorrhizal infection in heavy metal resistance. New Phytol. 91: 197-209.

Breuninger, M., Requena, N., 2004. Recognition events in AM symbiosis: analysis of fungal gene expression at the early appressorium stage. Fungal Genet. Biol. 41(8): 794-804.

Broadley, M.R., Willey, N.J., Mead, A., 1999. A method to assess taxomonic variation in shoot caesium concentration among flowering plants. Environ. Pollut. 106: 341-349.

Broadley, M.R., Escobar-Gutiérrez, A.J., Bowen, H.C., Willey, N.J., White, P.J., 2001. Influx and accumulation of Cs^+ by the atk1 mutant

of *Arabidopsis thaliana* (L.) Heynh. Lacking a dominant K^+ transport system. J. Exp. Bot. 52: 839-844.

Brückmann, A. & Wolters, V. 1994. Microbial immobilisation and recycling of 137Cs in the organic layers of forest ecosystems: relationship to environmental conditions, humification and invertebrate activity. Sci. Total Environ. 157: 249-256.

Brundrett, M.C., 2002. Coevolution of roots and mycorrhizas of land plants. New Phytol. 154 (2): 275-304.

Brunner, I., Beat, F., Riesen, T.K., 1996. Influence of ectomycorrhization and cesium/potassium ratio on uptake and localization of cesium in Norway spruce seedlings. Tree Physiol. 16: 705-711.

Bücking, H., Beckmann, S. Heyser, W. Kottke, I., 1998. Elemental contents in vacuolar granules of ectomycorrhizal fungi measured by EELS and EDXS. A comparison of different methods and preparation techniques. Micron. 29: 53-61.

Bücking, H., Shachar-Hill, Y., 2005. Phosphate uptake, transport and transfer by the arbuscular mycorrhizal fungus *Glomus intraradices* is stimulated by increased carbohydrate availability. New Phytol. 165(3): 899-912.

Bunzl, K., Kracke, W., Schimmack, W., 1992. Vertical migration of Pu-239 + 240, Am-241 and Cs-137 fallout in a forest soil under spruce. Analyst 117: 469-474.

Burleigh, S.H., Harrison, M.J., 1997. A novel gene whose expression in *Medicago truncatula* roots is suppressed in response to colonization by vesicular-arbuscular mycorrhizal (VAM) fungi and to phosphate nutrition. Plant Mol. Biol. 34: 199-208.

Burleigh, S.H., Bechmann, I.E., 2002. Plant nutrient transporter regulation in arbuscular mycorrhizas. Plant Soil 244: 247-251.

Burleigh, S.H., Jakobsen, I., 2002. Mycorrhizal research on the move. Trends Plant Sci. 7: 6-7.

Burleigh, S.H., Kristensen, B.K., Bechmann, I.E., 2003. A plasma membrane zinc transporter from *Medicago truncatula* is up-regulated in roots by Zn fertilization, yet down-regulated by arbuscular colonization. Plant Mol. Biol. 52: 1077-1088.

Buysse, J., VandenBrande, K., Merckx, R., 1996. Genotypic differences in the uptake and distribution of radiocaesium in plant. Plant Soil 178: 265-271.

Byrne, A.R., Ravnik, V., Kosta, L., 1976. Trace element concentrations in higher fungi. Science Total Environ. 6: 65-78.

Callow, J.A., Capaccio, L.C.M., Parish, G., Tinker, P.B., 1978. Detection and estimation of polyphosphate in vesicular-arbuscular mycorrhizas. New Phytol. 80: 125-134.

Camps, M., Rigol, A., Vidal, M., Rauret, G., 2003. Assessment of the suitability of soil amendments to reduce Cs-137 and Sr-90 root uptake in meadows. Environ. Sci. Technol. 37(12): 2820-2828.

Carter, M.W., 1993. Radionuclides in the food chain. Springler-Verlag, New York.

Cawse, P.A., 1983. The accumulation of caesium-137 and plutonium-239+240 in soils of Great Britain, and transfer to vegetation, in: Coughtrey, P.J., Bell, J.N.B., Roberts, T.M. (Eds.), Ecological aspects of radionuclide release. Blackwell Scientific Publications, Oxford, pp. 47-62.

Chalot, M., Brun, A., 1998. Physiology of organic nitrogen acquisition by ectomycorrhizal fungi and ectomycorrhizas, FEMS Microbiol. Rev. 22: 21-44.

Chapin, F.S., Matson, P.A., Mooney, H.A., 2002. Principles of Terrestrial Ecosystem Ecology. Springer Verlag, New York.

Chen, B.D., Jakobsen, I., Roos, P., Borggaard, O.K., Zhu, Y.G., 2005. Mycorrhiza and root hairs enhance acquisition of phosphorus and uranium from phosphate rock but mycorrhiza decreases root to shoot uranium transfer. New Phytol. 165: 591-598.

Christmann, A., Moes, D., Himmelbach, A., Tang, Y., Grill, E., 2006. Integration of abscisic acid signalling into plant responses. Plant Biol. 8(3): 314-325.

Clark, R.B., Zeto, S.K., 2000. Mineral acquisition by arbuscular mycorrhizal plants. J. Plant Nutr. 23: 867-902.

Cline, J.F., Hungate, F.P., 1960. Accumulation of potassium, caesium-137 and rubidium-86 in bean plants grown in nutrient solutions. Plant Physiol. 35: 826-829.

Clint, G.M., Dighton, J., 1992. Uptake and accumulation of radiocaesium by mycorrhizal and non-mycorrhizal heather plants. New Phytol. 121: 555-561.

Cole, L., Orlovich, D.A., Ashford, A.E., 1998. Structure, function, and motility of vacuoles in filamentous fungi. Fungal Genet. Biol. 24: 86-100.

Colpaert, J.V., Vandenkoornhuyse, P., Adriaensen, K., Vangronsveld, J., 2000. Genetic variation and heavy metal tolerance in the

ectomycorrhizal basidiomycete *Suillus luteus*. New Phytol. 147: 367-379.

Colpaert, J.V., Adriaensen, K., Muller, L.A.H., Lambaerts, M., Faes, C., Carleer, R., Vangronsveld, J., 2005. Element profiles and growth in Zn-sensitive and Zn-resistant Suilloid fungi. Mycorrhiza 15(8): 628-634.

Cooper, K.M., Tinker, P.B., 1978. Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas. II. Uptake and translocation of phosphorus, zinc and sulphur. New Physiol. 81: 43-52.

Cooper, K.M., Tinker, P.B., 1981. Translocation and transfer of nutrients in vesicular–arbuscular mycorrhizas. IV. Effect of environmental variables on movement of phosphorus. New Phytol. 88: 327-339.

Corratgé, C., Lambilliotte, R., Sentenac, H., Zimmermann, S., 2006. Potassium transporter and potassium channel of the ectomycorrhizal fungus *Hebeloma cylindrosporum* are candidates for symbiotic nutrient exchange, in: Barea, J.M., Azcón, C., Gutiérrez, F., Gonzàlez, F., Molina, A.J. (Eds.), 5th International conference on Mycorrhiza "Mycorrhiza for science an society", 23-27, July 2006, p 47.

Coughtrey, P.J., Thorne, M.C., 1983. Radionuclide distribution and transport in terrestrial and aquatic ecosystems – A critical review of data, Volume 1., Balkema, A.A., Rotterdam.

Courbot, M., Diez, L., Ruotolo, R., Chalot, M., Leroy, P., 2004 Cadmium-responsive thiols in the ectomycorrhizal fungus *Paxillus involutus*. Appl. Environ. Microbiol. 70: 7413-7417.

Cox, G., Moran, K.J., Sanders, F., Nockolds, C., Tinker, P.B., 1980. Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas. New Phytol. 84: 649-659.

Cranenbrouck, S., Voets, L., Bivort, C., Renard, L., Strullu, D.G., Declerck, S., 2005. Methodologies for successful aseptic growth of arbuscular mycorhizal fungi on root-organ cultures, in: Declerck, S., Strullu, D.-G., Fortin, J.A. (Eds.), In vitro culture of mycorrhizas. Springer-Verlag, Heidelberg, pp. 341-375.

Cumming, J.R., Ning, J., 2003. Arbuscular mycorrhizal fungi enhance aluminium resistance of broomsedge (*Andropogon virginicus* L.). J. Exp. Bot. 54: 1447-1459.

Daft, M.J., Nicolson, T.H., 1966. Effect of Endogone mycorrhiza on plant growth. New Phytol. 65: 343-350.

Danneberg, G., Latus, C., Zimmer, W., Hundeshagen, B., Schneiderpoetsch, H., Bothe, H., 1993. Influence of vesicular-arbuscular mycorrhiza on phytohormone balances in maize (*Zea mays* L). J. Plant Physiol. 141(1): 33-39.

Davies, W.J., Jones, H.G., 1991. Abscisic Acid. Bios Scientific Publishers Ltd., Oxford.

Declerck, S., Strullu, D.G., Plenchette, C., 1998. Monoxenic culture of the intraradical forms of Glomus sp. isolated from a tropical ecosystem: a proposed methodology for germplasm collection. Mycologia 90: 579-585.

Declerck, S., Leyval, C., Jakobsen, I., Thiry, Y., Heine, C., Delvaux, B., 2001a. The European project MYRRH: use of mycorrhizal fungi for the phytostabilisation of radio-contaminated environments. Radioprotection 37: 337-339.

Declerck, S., D'Or, D., Cranenbrouck, S., Le Boulengé, E., 2001b. Modelling the sporulation dynamics of arbuscular mycorrhizal fungi in monoxenic culture. Mycorrhiza 11: 225-230.

Declerck, S., Dupré de Boulois, H., Bivort, C., Delvaux, B., 2003. Extraradical mycelium of the arbuscular mycorrhizal fungus Glomus lamellosum can take up, accumulate and translocate radiocaesium under root-organ culture conditions. Environ. Microbiol. 5: 510-516.

Declerck, S., Strullu, D.G., Fortin, J.A., 2005. In vitro culture of mycorrhizas. Springer-Verlag, Heidelberg.

Delvaux, B., Kruyts, N., Cremers, A., 2000. Rhizosperic mobilization of radiocaesium in soils. Environ. Sci. Technol. 34: 1489-1493.

Delvaux, B., Kruyts, N., Maes, E., Smolders, E., 2001. Fate of radiocesium in soil and rhizosphere, in. Gobran, G.R., Wenzel, W.W., Lombi, E. (Eds.), Trace elements in the rhiszosphere. CRC Press, Bocan Raton, pp. 61-91.

Desmet, G., Nassimbeni, P., Belli, M., 1990. Transfer of radionuclides in natural and semi-natural environments. Elvesier Applied Science, London.

Dighton, J., Clint, G.M., Poskitt, J., 1991. Uptake and accumulation of 137Cs by upland grassland soil fungi: a potential pool of Cs immobilisation. Mycol. Res. 95(9): 1052-1056.

Dighton, J., Terry, G.M., 1996. Uptake and immobilization of caesium in UK grassland and forest soils by fungi following the Chernobyl accident, in Frankland, J.C., Magan, N., Gadd, G.M. (Eds), Fungi and environmental change. Cambridge University Press, Cambridge, pp. 184-200.

Doner, L.W., Bécard. G., 1991. Solubilization of gellan gels by chelation of cations. Biotechnol. Tech. 5: 25-29.

Drissner, J., Bürmann, W., Enslin, F., Heider, R., Klemt, E., Miller, R., Schick, G., Zibold, G., 1998. Availability of caesium radionuclides to plants-classification of soils and role of mycorrhiza. J. Environ. Radioactiv. 41(1): 19-32.

Driver, J.D., Holben, W.E., Rillig, M.C., 2005. Characterization of glomalin as a hyphal wall component of arbuscular mycorrhizal fungi. Soil Biol. Biochem. 37: 101-106.

Dupré de Boulois, H., Leyval, C., Joner, E.J., Jakobsen, I., Chen, B., Roos, P., Thiry, I., Rufyikiri, G., Devaux, B., Declerck, S., 2005a. Use of mycorrhizal fungi for the phytostabilisation of radio-contaminated environment (European project MYRRH): Overview on the scientific achievements. Radioprotection 40(1): S41-S46.

Dupré de Boulois, H., Delvaux, B., and Declerck, S., 2005b. Effects of arbuscular mycorrhizal fungi on the root uptake and translocation of radiocaesium. Environ. Pollut. 134(3): 515-524.

Dupré de Boulois, H., Voets, L., Delvaux, B., Jakobsen, I., Declerck, S., 2006a. Transport of radiocaesium by arbuscular mycorrhizal fungi to *Medicago truncatula* under *in vitro* conditions. Environ. Microbiol. 8(11): 1926-1934.

Dupré de Boulois, H., Declerck, S., 2006b. Transport of radiocaesium by arbuscular mycorrhizal fungi: from myths to reality, in: Barea, J.M., Azcón, C., Gutiérrez, F., Gonzàlez, F., Molina, A.J. (Eds.), 5th International conference on Mycorrhiza "Mycorrhiza for science an society", 23-27, July 2006, p. 157.

Dupré de Boulois, H., Voets, L., Declerck, S. *In vitro* compartmented systems to study transport in arbuscular mycorrhizal symbiosis, in: Varma, A. (Ed.), Mycorrhiza Manual, second ed., Springer-Verlag, Heidelberg, accepted for publication.

Durell, S.R., Hao, Y.L., Nakamura, T., Bakker, E.P., Guy, H.R., 1999. Evolutionary relationship between K^+ channels and symporters. Biophys. J. 77(2): 775-788.

Dushenkov, S., 2003. Trends in phytoremediation of radionuclides. Plant Soil 249: 167-175.

Eapen, S., Suseelan, K.N., Tivarekar, S., Kotwal, S.A., Mitra, R., 2003. Potential for rhizofiltration of uranium using hairy root cultures of *Brassica juncea* and *Chenopodium amaranticolor*. Environ. Res. 91: 127-133.

Ehlken, S., Kirchner, G., 2002. Environmental processes affecting plant root uptake of radioactive trace elements and variability of transfer factor data: a review. J. Environ. Radioactiv. 58: 97-112.

Elmeskaoui, A., Damont, J.P., Poulin, M.J., Piché, Y., Desjardins, Y., 1995. A tripartite culture system for endomycorrhizal inoculation of micropropagated strawberry plantlets *in vitro*. Mycorrhiza 5: 313-319.

Entry, J.A., Vance, N.C., Hamilton, M.A., Zabowski, D., Watrud, L.S., Adriano, D.C., 1996. Phytoremediation of soil contaminated with low concentrations of radionuclides. Water Air Soil Poll. 88: 167-176.

Entry, J.A., Astrud, L.S., Reeves, M., 1999. Accumulation of ^{137}Cs and ^{90}Sr from contaminated soil by three grass species inoculated with mycorrhizal fungi. Environ. Pollut. 104: 449-457.

Entry, J.A., Rygiewicz, P.T., Watrud, L.S., Donnelly, P.K., 2002. Influence of adverse soil conditions on the formation and function of Arbuscular mycorrhizas. Adv. Envion. Res. 7: 123-138

Ezawa, T., Smith, S.E., Smith, F.A., 2002. P metabolism and transport in AM fungi. Plant Soil 244: 221-230.

Ferrol, N., Barea, J.M., Azcon-Aguilar, C., 2002. Mechanisms of nutrient transport across interfaces in arbuscular mycorrhizas. Plant Soil 244 (1-2): 231-237.

Fillion, M., St-Arnaud, M., Fortin, J.A., 1999. Direct interaction between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere micro-organisms. New Phytol. 141: 525-533.

Fortin, J. A., Bécard, G., Declerck, S., Dalpé, Y., St Arnaud, M., Coughlan, A. P., Piché, Y., 2002. Arbuscular mycorrhiza on root-organ cultures. Can. J. Bot. 80: 1-20.

Foster, R., Chua, N.H., 1999. An Arabidopsis mutant with deregulated ABA gene expression: implications for negative regulator function. Plant J. 17(4): 363-372.

Frey, B., Schuepp, H., 1992. Transfer of symbiotically fixed nitrogen from berseen (*Trifolium alexandrinum* L.) to maize via vesicular-arbuscular mycorrhizal hyphae. New Phytol. 122: 447-454.

Frey, B., Zierold, K., Brunner, I., 2000. Extracellular complexation of Cd in the Hartig net and cytosolic Zn sequestration in the fungal mantle of *Picea abies–Hebeloma crustuliniforme* ectomycorrhizas. Plant Cell Environ. 23: 1257-1265.

Frissel, M.J., Deb, D.L., Fathony, M., Lin, Y.M., Mollah, A.S., Ngo, N.T., Othman, I., Robinson, W.L., Skarlou-Alexiou, V., Topcuoglu, S., Twining, J.R., Uchida, S., Wasserman, M.A., 2002. Generic values for soil-to-plant transfer factors of radiocesium. J. Environ.Radioactiv. 58: 113-128.

Gadd, G.M., 1993. Interactions of fungi with toxic metals, New Phytol. 124: 25-60.

Gadd, G.M., 1996. Influence of microorganisms on the environmental fate of radionuclides. Endeavour 20: 150-156.

Gadd, G.M., 2004. Microbial influence on metal mobility and application for bioremediation. Geoderma 122: 109-119.

Galli, U., Schuepp, H., Brunold, C., 1994. Heavy metal binding by mycorrhizal fungi. Physiol. Plant 92: 364-368.

Gassmann, W., Rubio, F., Schroeder, J.I., 1996. Alkali cation selectivity of the wheat root high-affinity potassium transporter HKT1. Plant J. 10: 869-882.

Gaur, A., Adholeya, A., 2004. Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Current Sci. 86: 528-534.

Gaymard, F., Pilot, G., Lacombe, B., Bouchez, D., Bruneau, D., Boucherez, J., Michaux-Ferriere, N., Thibaud, J.B., Sentenac, H., 1998. Identification and disruption of a plant shaker-like outward channel involved in K^+ release into the xylem sap. Cell 94(5): 647-655.

Gentry, T.J., Rensing, C., Pepper, I.L., 2004. New approaches for bioaugmentation as a remediation technology. Crit. Rev. Environ. Sci. Technol. 34: 447-494.

George, E., Häussler, K., Vetterlein, D., Gorgus, E., Marschner, H.., 1992. Water and nutrient translocation by hyphae of *Glomus mosseae*. Can. J Bot. 70: 2130-2137.

Gianinazzi-Pearson, V., 1996. Plant cell responses to arbuscular mycorrhizal fungi: getting to the root of the symbiosis. Plant Cell 8: 1871-1883.

Gillett, A.G., Crout, N.M.J., 2000. A review of ^{137}Cs transfer to fungi and consequences for modelling environmental transfer. J. Environ. Radioact. 48: 95-121.

Gilliham, M., Tester, M., 2005. The regulation of anion loading to the maize root xylem. Plant Physiol. 137: 819-828.

Giovannetti, M., Mosse. B., 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84: 489-500.

Giovannetti, M., Azzolini, D., Citernesi, A.S., 1999. Anastomosis formation and nuclear and protoplasmic exchange in arbuscular mycorrhizal fungi. Appl. Environ. Microb. 65: 5571-5575.

Göhre, V., Paszkowski, U., 2006. Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. Planta 223 (6): 1115-1122.

Gommers, A. 2001. Radiocaesium uptake and cycling in willow short rotation coppice. PhD thesis, Faculty of Agricultural and Applied Biological Sciences, Katholieke Universiteit Leuven, Belgium.

González-Chávez, C., D'Haen, J., Vangronsveld, J., Dodd, JC., 2002. Copper sorption and accumulation by the extraradical mycelium of different Glomus spp. (arbuscular mycorrhizal fungi) isolated from the same polluted soil. Plant Soil 240: 287-297.

González-Chávez, M.C., Carrillo-González, R., Wright, S.F., Nichols, K.A., 2004. The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. Environ. Pollut. 130: 317-323.

Govindarajulu, M., Pfeffer, P.E., Jin, H.R., Abubaker, J., Douds, D.D., Allen, J.W.B., Bücking, H., Lammers, P.J., Shachar-Hill, Y., 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435: 819-823.

Gray, S.N., 1998. Fungi as potential bioremediation agents in soil contaminated with heavy or radioactive metals. Biochem. Soc. Trans. 26(4): 666-670.

Gray, S.N., Dighton, J., Olsson, S., Jennings, D., 1995. Real-time measurement of uptake of ^{137}Cs within mycelium of *Schizophyllum commune* Fr. by autoradiography followed by quantitative image analysis. New Phytol. 129: 449-465.

Grayston, S.J., Vaughan, D. Jones, D., 1996. Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Appl. Soil Ecol. 5: 29-56.

Grueter, H., 1971. Radioactive fission product ^{137}Cs in mushrooms in W. Germany during 1963–1970. Health Phys. 20: 655-656.

Guilitte, O., Melin, J., Wallberg, L., 1994. Biological pathways of radionuclides originating from the Chernobyl fallout in a boreal forest ecosystem. Sci. Total Environ. 157: 207-215.

Hampton, C.R., Bowen, H.C., Broadley, M.R., Hammond, J.P., Mead, A., Payne, K.A., Pritchard, J., White, P.J., 2004. Cesium toxicity in Arabidopsis. Plant Physiol. 136: 3824-3837.

Hampton, C.R., Broadley, M.R., White, P.J., 2005. Short review: the mechanisms of radiocaesium uptake by *Arabidopsis* roots. Nukleonika 50: S3-S8.

Harley, J.L., 1969. The biology of mycorrhiza, 2nd edn. Leonard Hill, London.

Haro, R., Rodríguez-Navarro, A., 2002. Molecular analysis of the mechanism of potassium uptake through the TRK1 transporter of Saccharomyces cerevisiae. BBA-Biomembranes 1564(1): 114-122.

Harrison, M., 2005. Signaling in the arbuscular mycorrhizal symbiosis. Annu. Rev. Microbiol. 59: 19-42.

Hartley, J., Cairney, J.W.G., Meharg, A.A., 1997. Do ectomycorrhizal fungi exhibit tolerance to potentially toxic metals in the environment? Plant Soil 189: 303-319.

Hattingh, M.J., Gray, L.E., Gerdemann, J.M., 1973. Uptake and translocation of 32P-labelled phosphate to onion roots by endomycorrhizal fungi. Soil Sci. 166: 383-387.

Hawkins, H.J., Johansen A, George, E., 2000. Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. Plant Soil 226(2): 275-285.

Hayman, D.S., Mosse, B., 1972. Plant growth response to vesicular-arbuscular mycorrhiza. III. Increased uptake of labile P from soil. New Phytol. 71: 41-47.

Hentschel, E., Godbold, D.L., Marschner, P., Schlegel, H., Jentschke, G., 1993. The effect of *Paxillus involutus* Fr. on aluminium sensitivity of Norway spruce seedlings. Tree Physiol. 12: 379-390.

Hernández-Sebastià, C., Piché, Y., Desjardins, Y., 1999. Water relations of whole strawberry plantlets *in vitro* inoculated with *Glomus intraradices* in a tripartite culture system. Plant Sci. 143: 81-91.

Hernández-Sebastià, C., Samson, G., Bernier, P.Y., Piché, Y., Desjardins, Y., 2000. *Glomus intraradices* causes differential changes in amino acid and starch concentrations of *in vitro* strawberry subjected to water stress. New Phytol. 148: 177-186.

Hoth, S., Morgante, M., Sanchez, J.P., Hanafey, M.K., Tingey, S.V., Chua, N.H., 2002. Genome-wide gene expression profiling in *Arabidopsis thaliana* reveals new targets of abscisic acid and largely

impaired gene regulation in the abi1-1 mutant. J. Cell Sci. 115 (24): 4891-4900.

Howard, B.J., Beresford, N.A., Hove, K., 1991. Transfer of radiocaesium to ruminants in natural and semi-natural ecosystems and appropriate countermeasures. Health Phys. 61: 715-725.

Jakobsen, I., 1999. Transport of phosphorus and carbon in arbuscular mycorrhizas, in: Varma, A., Hock, B. (Eds.), Mycorrhiza. Structure, function, molecular biology and biotechnology. Springer-Verlag, Berlin, pp. 305-332.

Jansa, J., Mozafar, A., Frossard, E., 2003. Long-distance transport of P and Zn through the hyphae of an arbuscular mycorrhizal fungus in symbiosis with maize. Agronomie 23 (5-6): 481-488.

Jentschke, G., Goldbold, D.L., 2000. Metal toxicity and ectomycorrhizas. Physiol. Plant 109: 107-116.

Jentschke, G., Winter, S., Godbold, D.L., 1999. Ectomycorrhizas and cadmium toxicity in Norway spruce seedlings. Tree Physiol. 19: 23-30.

Jentschke, G., Brandes, B., Kuhn, A.J., Schröder, W.H., Goldbold, D.L., 2001. Interdependence of phosphorus, nitrogen, potassium and

magnesium translocation by the ectomycorrhizal fungus Paxillus involutus. New Phytol. 149: 327-337.

Jin, H., Pfeffer, P.E., Douds, D.D., Piotrowski, E., Lammers, P.J., Shachar-Hill, Y., 2005. The uptake, metabolism, transport and transfer of nitrogen in an arbuscular mycorrhizal symbiosis. New Phytol. 168: 687-696.

Joner, E.J., Leyval, C., 1997. Uptake of ^{109}Cd by roots and hyphae of a Glomus mosseae/Trifolium subterraneum mycorrhiza from soil amended with high and low concentrations of cadmium. New Phytol. 135: 353-360.

Joner, E.J., Briones, R., Leyval, C., 2000a. Metal binding capacity of arbuscular mycorrhizal mycelium. Plant Soil 226: 227-234.

Joner, E.J., Ravnskov, S., Jakobsen, I., 2000b. Arbuscular mycorrhizal phosphate transport under monoxenic conditions using radio-labelled inorganic and organic phosphate. Biotechnol. Lett. 22: 1705-1708.

Joner, E.J., Roos, P., Jansa, J., Frossard, E., Leyval, C., Jakobsen, I., 2004. No significant contribution of arbuscular mycorrhizal fungi to transfer of radiocesium from soil to plants. Appl. Environ. Microb. 70(11): 6512-6517.

Jones, D.L., Hodge, A,. Kuzyakov, Y., 2004. Plant and mycorrhizal regulation of rhizodeposition. New Phytol. 163: 459-480.

Jones, M.D., Hutchinson, T.C., 1988. Nickel toxicity in mycorrhizal birch seedlings infected with Lactarius rufus or *Scleroderma flavidum*. II. Uptake of nickel, calcium, magnesium phosphorus and iron. New Phytol. 108: 461-470.

Kalač, P., 2001. A review of edible mushroom radioactivity. Food Chem. 75: 29-35.

Kaldorf, M., Kuhn, A.J., Schröder, W.H., Hildebrandt, U., Bothe, H., 1999. Selective element deposits in maize colonized by a heavy-metal tolerance conferring arbuscular mycorrhizal fungus. J. Plant Physiol. 154: 718-728.

Ketchum, K.A., Joiner, W.J., Sellers, A.J., Kaczmarek, L.K., Goldstein, S.A., 1995. A new family of outwardly rectifying potassium channel proteins with two pore domains in tandem. Nature 376: 690-695.

Kim, E.J., Kwak, J.M., Uozumi, N., Schroeder, J.I., 1998. AtKUP1: an Arabidopsis gene encoding high-affinity potassium transport activity. Plant Cell 10: 51-62.

Koch, K., 2004. Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. Curr. Opin. Plant Biol. 7: 235-246.

Kochian, L.V., Hoekenga, O.A., Pineros, M.A., 2004. How do crop plants tolerate acid soils? – Mechanisms of aluminum tolerance and phosphorous efficiency. Ann Rev. Plant Biol. 55: 459-493.

Köhler, B., Raschke, K., 2000. The delivery of salts to the xylem. Three types of anion conductance in the plasmalemma of the xylem parenchyma of roots of barley. Plant Physiol. 122: 243-254.

Korobova, E., Ermakov, A., Linnik, V., 1998. Cs-137 and Sr-90 mobility in soils and transfer in soil-plant systems in the Novozybkov district affected by the Chernobyl accident. Appl. Geochem. 13: 803-814.

Krupa, P., Kozdroj, J., 2004. Accumulation of heavy metals by ectomycorrhizal fungi colonizing birch trees growing in an industrial desert soil. World J. Microb. Biot. 20(4): 427-430.

Kruyts, N., Delvaux, B., 2002. Soil organic horizons as a major source for radiocesium biorecycling in forest ecosystems. J. Environ. Radioactiv. 58: 175-190.

Kuchenbuch, R., Claassen, N., Jungk A., 1986. Potassium availability in relation to soil moisture. I. Effect of soil moisture on potassium diffusion, root growth and potassium uptake of onion plants. Plant Soil 95(2): 221-231

Kuiper, I., Lagendijk, E.L., Bloemberg, G.V., Lugtenberg, B.J., 2004. Rhizoremediation: a beneficial plant–microbe interaction. Mol. Plant Microbe Interact. 17: 6-15.

Lacombe, B., Pilot, G., Gaymard, F., Sentenac, H., Thibaud, J.B., 2000. pH control of the plant outwardly-rectifying potassium channel SKOR. FEBS Lett. 466: 351-354.

Landeweert, R., Hoffland, E., Finlay, R.D., Kuyper, T.W., Van Breemen, N., 2001. Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. Trends Ecol. Evol. 16: 248-254.

Lanfranco, L., Bolchi, A., Ros, E.C., Ottonello, S., Bonfante, P., 2002. Differential expression of a metallothionein gene during the presymbiotic versus the symbiotic phase of an arbuscular mycorrhizal fungus. Plant Physiol. 130: 58-67.

Leigh, R.A., Wyn Jones, R.G., 1984. A hypothesis relating critical potassium concentrations for growth to the distribution and functions of this ion in the plant cell. New Phytol. 97: 1-13.

Lembrechts, J. 1993. A review of literature on the effectiveness of chemical amendments in reducing the soil-to-plant transfer of radiostrontium and radiocaesium. Sci. Total Environ. 137: 81-98.

Leyval, C., Turnau, K., Haselwandter, K., 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7: 139-153.

Leyval, C., Joner, E.J., del Val, C., Haselwandter, K., 2002. Potential of arbuscular mycorrhizal fungi for bioremediation, in: Gianinazzi, S., Schüepp, H., Barea, J.M., Haselwandter, K. (Eds.), Mycorrhizal Technology in Agriculture. Birkhaüser Verlag, Basel, Switzerland, pp. 263-285.

Lin W., 1981. Inhibition of anion transport in corn root protoplasts. Plant Physiol. 68: 435-438.

Livens, F.R., Rimmer, D.L., 1988. Physico-chemical controls on artificial radionuclides in soil. Soil Use Manage. 4: 63-69.

Liu, A., Hamel, C., Elmi, A., Costa, C., Ma, B., Smith, D.L., 2002. Concentrations of K, Ca and Mg in maize colonized by arbuscular mycorrhizal fungi under field conditions. Can. J. Soil Sci. 82(3): 271-278.

Lloyd, J.R., Renshaw, J.C., 2005. Bioremediation of radioactive waste: radionuclide–microbe interactions in laboratory and field-scale studies. Curr. Opin. Biotechnol. 16: 254-260.

Louche-Tessandier, D., Samson, G., Hernández-Sebastià, C., Chagvardieff, P., Desjardins, Y., 1999. Importance of light and CO_2 on the effects of endomycorrhiza colonization on growth and photosynthesis of potato plantlets (*Solanum tuberosum*) in an *in vitro* tripartite system. New Phytol. 142: 539-550.

Ludwig-Müller, J., 2000. Hormonal balance in plants during colonization by mycorrhizal fungi, in: Kapulnik, Y., Douds Jr, D.D. (Eds.), Arbuscular mycorrhizas: physiology and function. Kluwer Academic Publishers, Dordrecht, pp. 263-285.

Maathuis, F.J.M., Sanders, D., 1997. Regulation of K^+ absorption in plant root cells by external K^+: interplay of different plasma membrane K^+ transporters. J. Exp. Bot. 48: 451-458.

Mäder, P., Vierheilig, H., Alt, M., Wiemken, A., 1993. Boundaries between soil compartments formed by microporous hydrophobic membranes (GORE-TEX®) can be crossed by vesicular-arbuscular mycorrhizal fungi but not by ions in the soil solution. Plant Soil 159: 201-206.

Maldonado-Mendoza, I.E., Dewbre, G.R., Harrison, M.J., 2001. A phosphate transporter gene from the extraradical mycelium of an arbuscular mycorrhizal fungus *Glomus intraradices* is regulated in response to phosphate in the environment. Mol. Plant-Microbe Interact. 14: 1140-1148.

Marschner, H., 1995. Mineral nutrition of higher plants. Academic Press, London.

Marschner, P., Baumann, K., 2003. Changes in bacterial community structure induced by mycorrhizal colonization in split-root maize. Plant Soil 251: 279-289.

Martino, E., Perotto, S., Parsons, R., Gadd, G.M., 2003. Solubilization of insoluble inorganic zinc compounds by ericoid mycorrhizal fungi derived from heavy metal polluted sites. Soil Biol. Biochem. 35: 133-141.

Mc Graw, A.C., Gamble, J.F., Schenck, N.C., 1979. Vesicular-arbuscular mycorrhizal uptake of Cs-134 in two tropical pasture grass species. Phytopathology 69: 1038-1041.

McGee, E.J., Synnott, H.J., Johansson, K.J., Fawaris, B.H., Nielsen, S.P., Horrill, A.D., Kennedy, V.H., Barbayiannis, N., Veresoglou, D.S., Dawson, D.E., Colgan, P.A., McGarry, A.T., 2000. Chernobyl

fallout in a Swedish spruce forest ecosystem. J. Environ. Radioactiv. 48: 59-78.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A., 1990. A new Method which gives an objective measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. New Phytol. 115(3): 495-501.

Meharg, A.A., 2003. The mechanistic basis of interactions between mycorrhizal associations and toxic metal cations. Mycol. Res. 107: 1253-1265.

Meharg, A.A., Cairney, J.W.G., 2000. Ectomycorrhizas - extending the capabilities of rhizosphere remediation? Soil Biol. Biochem. 32: 1475-1484.

Meixner, C., Ludwig-Muller, J., Miersch, O., Gresshoff, P., Staehelin, C., Vierheilig, H., 2005. Lack of mycorrhizal autoregulation and phytohormonal changes in the supernodulating soybean mutant nts1007. Planta 222(4): 709-715.

Mosse, B., Hepper, C.M., 1975. Vesicular-arbuscular infections in root-organs cultures. Physiol. Plant Pathol. 5: 215-223.

Newman, E.I., 1966. A method of estimating the total lenght of root in a sample. J. Appl. Ecol. 3: 139-145.

Nielsen, J.S., Joner, E.J., Declerck, S., Olsson, S., Jakobsen, I., 2002. Phospho-imaging as a tool for visualization and noninvasive measurement of P transport dynamics in arbuscular mycorrhizas. New Phytol. 154: 809-820.

Nies, D.H., 1999. Microbial heavy-metal resistance. Appl. Microbiol. Biotechnol. 51: 730-750.

Nikolova, I., Johanson, K.J., Dahlberg, A., 1997. Radiocaesium in fruitbodies and mycorrhizae in ectomycorrhizal fungi. J. Environ. Radioactiv. 37(1): 115-125.

Nishita, H., Raug, R.M., Hamilton, M., 1968. Influence of minerals on Sr90 and Cs137 uptake by bean plants. Soil Science 105: 237-243.

Olsen, R.A. 1994. The transfer of radiocaesium from soil to plants and fungi in seminatural ecosystems. Nordic Radioecology, in: Dahlgaard, H. (ed.), The transfer of radionuclides through Nordic ecosystems to man. Elsevier, Amsterdam, pp. 265-287.

Orlovich, D.A., Ashford, A.E., 1993. Polyphosphate granules are an artefact of specimen preparation in the ectomycorrhizal fungus *Pisolithus tinctorius*. Protoplasma 173: 91-102.

Pan, W.L., Hopkins, A.G., Jackson, W.A., 1989. Aluminium inhibition of shoot lateral branches of *Gycine max* and reversal by exogeneous cytokinin. Plant Soil 120: 1-9.

Pawlowska, T.E., Charvat, I., 2004. Heavy-metal stress and developmental patterns of arbuscular mycorrhizal fungi. Appl. Environ. Microbiol. 70: 6643-6649.

Payne, K.A., Bowen, H.C., Hammond, J.P., Hampton, C.R., Lynn, J.R., Mead, A., Swarup, K., Bennett, M.J., White, P.J., Broadley, M.R., 2004. Natural genetic variation in caesium (Cs) accumulation by *Arabidopsis thaliana*. New Phytol. 162: 535-548.

Pearson, V., Tinker, P.B., 1975. Measurement of phosphorus fluxes in the external hyphae of endomycorrhizas, in: Sanders, F.E., Mosse, B., Tinker, P.B., (eds.), Endomycorrhizas. Academic Press, London & New York, pp. 277-287.

Pearson, J.N., Jakobsen, I., 1993. The relative contribution of hyphae and roots to phosphorus uptake by arbuscular mycorrhizal plants, measured by dual labeling with ^{32}P and ^{33}P. New Phytol. 124(3): 489-494.

Peuke, A.D., Jeschke, W.D., Hartung, W., 2002. Flows of elements, ions and abscisic acid in *Ricinus communis* and site of nitrate reduction under potassium limitation. J. Exp. Bot. 53: 241-250.

Pfeffer, P., Douds, D.D., Bucking, H., Schwartz, D.P., Shachar-Hill, Y. 2004. The fungus does not transfer carbon to or between roots in an arbuscular mycorrhizal symbiosis. New Phytol. 163: 617-627.

Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55: 158-161.

Pilot, G., Gaymard, F., Mouline, K., Chérel, I., Sentenac, H., 2003. Regulated expression of Arabidopsis Shaker K^+ channel genes involved in K^+ uptake and distribution in the plant. Plant Mol. Biol. 51: 773-787.

Plenchette, C., Morel, C., 1996. External phosphorus requirements of mycorrhizal and non-mycorrhizal barley and soybean plants. Biol. Fertil. Soils 21: 303-308.

Rafferty, B., Dawson, D., Kliashtorin, A., 1997. Decomposition in two pine forests: the mobilisation of 137Cs and K from forest litter. Soil Biol. Biochem. 29 (11-12): 1673-1681.

Rafferty B., Brennan, M., Dawson, D., Dowding, D., 2000. Mechanisms of Cs-137 migration in coniferous forest soils. J. Environ. Radioactiv. 48: 131-143.

Rapp, C., Jentschke G., 1994. Acid deposition and ectomycorrhizal symbiosis: Field investigations and causal relationships, in: Godbold, D.L., Hüttermann, A. (Eds.), Effects of Acid Rain on Forest Processes. Wiley, New York, pp. 183-230.

Rausch, C., Daram, P., Brunner, S., Jansa, J., Laloi, M., Leggewie, G., Amrhein, N., Bucher, M., 2001. A phosphate transporter expressed in arbuscule-containing cells in potato. Nature 414: 462-466.

Requena, N., Breuninger, M., Franken, P., Ocón, A., 2003. Symbiotic status, phosphate, and sucrose regulate the expression of two plasma membrane H^+-ATPase genes from the mycorrhizal fungus *Glomus mosseae*. Plant Physiol. 132(3): 1540-1549.

Rhodes, L.H., Gerdemann, J.W., 1975. Phosphate uptake zones of mycorrhizal and non-mycorrhizal onions. New Phytol. 75: 555-561.

Richter, C., Marschner, H., 1973. Turnover of potassium in different root zones of corn seedlings. Z. Pflanzenernahr. Bodenkd. 70: 211-221.

Riesen, T.K., Brunner, I., 1996. Effect of ectomycorrhizae and ammonium on ^{134}Cs and ^{85}Sr uptake into *Picea abies* seedlings, Environ. Pollut. 93: 1-8.

Rillig, M.C., Mummey, D.L., Ramsey, P.W., Klironomos, J.N., Gannon, J.E., 2006. Phylogeny of arbuscular mycorrhizal fungi predicts community composition of symbiosis-associated bacteria. FEMS Microbiol. Ecol. 57(3): 389-395.

Rillig, M.C., 2004. Arbuscular mycorrhizae and terrestrial ecosystem processes. Ecol. Lett. 7: 740-754.

Roberts, S.K., 1998. Regulation of K^+ channels in maize roots by water stress and abscisic acid. Plant Physiol. 116: 145-153.

Roberts, S.K., 2006. Plasma membrane anion channels in higher plants and their putative functions in roots. New Phytol. 169 (4): 647-666.

Roberts, S.K., Snowman, B.N., 2000. The effects of ABA on channel-mediated K^+ transport across higher plant roots. J. Exp. Bot. 51: 1585-1594.

Robison, W.L., Stone, E.L., 1992. The Effect of Potassium on the Uptake of 137Cs in Food Crops Grown on Coral Soils: Coconut at Bikini Atoll. Health Phys. 62: 496-511.

Rodríguez-Navarro, A., 2000. Potassium transport in fungi and plants. BBA-Biomembranes 1469(1): 1-30.

Rogers, R.D., Williams, S.E., 1986. Vesicular-arbuscular mycorrhiza: influence on plant uptake of cesium and cobalt. Soil Biol. Biochem. 18: 371-376.

Rommey, E.M., Neel, J.W., Nishita, H., Olafson, J.H., Larson, K.H., 1957. Plant uptake of Sr90, Y91, Ru106, Cs137, and Ce144 fron soils. Soil Sci. 83: 369-376.

Rosén, K., Öborn, I., Lönsjö, H., 1999. Migration of radiocaesium in Swedish soil profiles after the Chernobyl accident, 1987-1995. J. Environ. Radioactiv. 46: 45-66.

Rosén, K., Zhong, W.L., and Mårtensson, A., 2005. Arbuscular mycorrhizal fungi mediated uptake of ^{137}Cs in leek and ryegrass. Sci. Total Environ. 338(3): 283-290.

Rufyikiri, G., Thiry, Y., Wang, L., Delvaux, B., Declerck, S., 2002. Uranium uptake and translocation by the arbuscular mycorrhizal fungus, Glomus intraradices, under root organ culture conditions. New Phytol. 156: 275-281.

Rufyikiri, G., Thiry, Y., Delvaux, B., Declerck, S., 2003. Contribution of hyphae and roots to uranium uptake, accumulation and translocation by arbuscular mycorrhizal carrot roots under root-organ culture conditions. New Phytol. 158: 391-399.

Rufyikiri, G., Declerck, S., Thiry, Y., 2004. Comparison of U-233 and P-33 uptake and translocation by the arbuscular mycorrhizal fungus *Glomus intraradices* in root organ culture conditions. Mycorrhiza 14(3): 203-207.

Rufyikiri, G., Kruyts, N., Declerck, S., Thiry, Y., Delvaux, B., Dupré de Boulois, H., Joner, E., 2005. Uptake, assimilation and translocation of mineral elements in monoxenic cultivation systems, in: Declerck, S., Strullu, D.-G., Fortin, J.A. (Eds.), In vitro culture of mycorrhizas. Springer-Verlag, Heidelberg, pp. 201-215.

Ryan, M.H., McCully, M.E., Huang, C.X., 2003. Location and quantification of phosphorus and other elements in fully hydrated, soil-grown arbuscular mycorrhizas: a cryo-analytical scanning electron microscopy study. New Phytol. 160(2): 429-441.

Sacchi, G.A., Espen, L., Nocito, F., Cocucci, M., 1997. Cs^+ uptake in subapical maize root segments: Mechanism and effects on H^+ release, transmembrane electric potential and cell pH. Plant Cell Physiol. 38(3): 282-289.

Salton, M.R.J., 1960. Microbial cell walls. John Wiley & Sons, Inc., New York.

Sanders, F.E., Tinker, P.B., 1971. Mechanism of absorption of phosphate from soil by Endogone mycorrhizas. Nature 233: 278-279.

Sanders, F.E., Tinker, P.B., 1973. Phosphate flow into mycorrhizal roots. Pesticide Science 4: 385-395

Santa-María, G.E., Rubio, F., Dubcovsky, J., Rodríguez-Navarro, A., 1997. The HAK1 gene of barley is a member of a large gene family and encodes a high-affinity potassium transporter. Plant Cell 9: 2281-2289.

Schachtman, D.P., Schroeder, J.I., 1994. Structure and transport mechanism of a high-affinity potassium uptake transporter from higher plants. Nature 370: 655-658.

Schachtman, D.P., Reid, R.J., Ayling, S.M., 1998. Phosphorus uptake by plants: From soil to cell. Plant Physiol. 116(2): 447-453.

Schachtman, D.P., Schroeder, J.I., Lucas, W.J., Anderson, J.A., Gaber, R.F., 1992. Expression of an inward-rectifying potassium channel by the Arabidopsis KAT1 cDNA. Science 258: 1654-1657.

Schmülling, T., Schäfer, S., Romanov, G., 1997. Cytokinins as regulators of gene expression. Physiol. Plant. 100(3): 505-519.

Schwab, S.M., Menge, J.A., Tinker, P.B., 1991. Regulation of nutrient transfer between host and fungus in vesicular-arbuscular mycorrhizas. New Phytol. 117: 387-398.

Schweiger, P., Jakobsen, I., 2000. Laboratory and field methods for measurement of hyphal uptake of nutrients in soil. Plant Soil 226(2): 237-244.

Shaw, G., Bell, J.N.B., 1994. Plants and radionuclides, in: Frago, M.E., (Ed.), Plants and chemical elements: biochemistry, uptake, tolerance and toxicity. VCH Publishers, Weinheim, pp. 179-220.

Shutov, V.N., Bruk, G.Y., Basalaeva, L.N., Vasilevitsky, V.A., Ivanova, N.P. Kaplun, I.S., 1996. The role of mushrooms and berries in the formation of internal exposure doses to the population of Russia after the Chernobyl accident. Radiat. Protect. Dosim. 67: 55-64.

Smith, F.A., Jakobsen, I., Smith, S.E., 2000. Spatial differences in acquisition of soil phosphate between two arbuscular mycorrhizal fungi in symbiosis with *Medicago truncatula*. New Phytol. 147(2): 357-366.

Smith, J.T., Elder, D.G., 1999. A comparison of models for characterizing the distribution of radionuclides with depth in soils. Eur. J. Soil Sci. 50: 295-307.

Smith, M.L., Taylor, H.W., Sharma, H.D., 1993. Comparison of the post-Chernobyl ^{137}Cs contamination of musbrooms from Eastern

Europe, Sweden and North America. Appl. Environ. Microbiol. 59: 134-139.

Smith, S.E., Gianinazzi-Pearson, V., 1988. Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 39: 221-244.

Smith, S.E., Read, D.J., 1997. Mycorrhizal symbiosis. Academic Press, San Diego.

Smith, S.E., Smith, F.A., Jakobsen, I., 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiol. 133(1): 16-20.

Smolders, E., Kiebooms, L., Buysse, J., Merckx, R., 1996. Cs-137 uptake in spring wheat (*Triticum aestivum* L. cv. Tonic) at varying K supply: I. The effect in soil solution culture. Plant Soil 181: 205-209.

Smolders, E., Van den Brande, K., Merckx. R., 1997. The concentration of Cs-137 ans K in soil solution predict the plant availability of Cs-137 in 30 different soils. Environ. Sci. Technol. 31: 3432-3438.

St-Arnaud, M., Hamel, C., Vimard, B., Caron, M., Fortin, J.A., 1995. Altered growth of *Fusarium oxysporum* f. sp. *chrysanthemi* in an *in vitro* dual culture system with the vesicular arbuscular mycorrhizal

fungus *Glomus intraradices* growing on *Daucus carota* transformed roots. Mycorrhiza 5: 431-438.

St-Arnaud, M., Hamel, C., Vimard, B., Caron, M., Fortin, J.A., 1996. Enhanced hyphal growth and spore production of the arbuscular mycorrhizal fungus *Glomus intraradices* in an *in vitro* system in the absence of host roots. Mycol. Res. 100: 328-332.

StatSoft Inc., 2001. Statistica® Release 6. Statsoft Inc, Tulsa.

Steiner, M., Linkov, I., Yoshida, S., 2002. The role of fungi in the transfer and cycling of radionuclides in forest ecosystems. J. Environ. Radioactiv. 58(2-3): 217-241.

Strandberg, M., Johansson, M., 1998. 134-Cs in heather seed plants grown with and without mycorrhiza. J. Environ. Radioactiv. 40: 175-184.

Strebl, F., Gerzabek, M.H., Bossew, P., Kienzl, K., 1999. Distribution of radiocaesium in an Austrian forest stand. Sci. Total Environ. 226: 75-83.

Strullu, D.G., Romand, C., 1986. Méthode d'obtention d'endomycorhizes à vésicules et arbuscules en conditions axéniques. C.R. Acad. Sci. Paris 303: 245-250.

Suresh B., Ravishankar, G.A., 2004. Phytoremediation - a novel and promising approach for environmental clean-up. Crit. Rev. Biotechnol. 24: 97-124.

Suzuki, H., Kumagai, H., Oohashi, K., Sakamoto, K., Inubushi, K., Enomoto, S., 2001. Transport of trace elements through the hyphae of an arbuscular mycorrhizal fungus into marigold determined by the multitracer technique. Soil Sci. Plant Nutr. 47 (1): 131-137.

Suzuki, Y., Banfield, J.F., 2004. Resistance to, and accumulation of, uranium by bacteria from a uranium-contaminated site. Geomicrobiol. J. 21: 113-121.

Tam, P.C.F., 1995. Heavy metal tolerance by ectomycorrhial fungi and metal amelioration by *Pisolithus tinctorius*. Mycorrhiza 5: 181-187.

Tanaka, Y., Yano, K., 2005. Nitrogen delivery to maize via mycorrhizal hyphae depends on the form of N supplied. Plant Cell Environ. 28(10): 1247-1254.

Tegen, I., Dörr, H., Münnich, K.O., 1991. Laboratory experiments to investigate influence of microbial activity on the migration of caesium in a forest soil. Water Air Soil Poll. 75(58): 441-447.

Terada, H., Shibata, H., Kato, F., Sugiyama, H., 1998. Influence of alkali elements on the accumulation of radiocesium by mushrooms. J. Radioanal. Nucl. Ch. 235(1-2): 195-200.

Tester, M., 1999. The control of long-distance K^+ transport by ABA. Trends Plant Sci. 4(1): 5-6.

Thirty, Y. & Myttenaere, C. 1993. Behaviour of radiocaesium in forest multilayered soils. J. Environ. Radioactiv. 18: 247-257.

Timmer, L.W., Leyden, R.F., 1980. The relationship of mycorrhizal infection to phosphorus-induced copper deficiency in sour orange seedlings. New Phytol. 85: 15-23.

Tobar, R., Azcon, R., Barea, J.M., 1994. Improved nitrogen uptake and transport from N-15-labeled nitrate by external hyphae of arbuscular mycorrhiza under water-stressed conditions. New Phytol. 126(1): 119-122.

Torelli, A., Trotta, A., Acerbi, l., Arcidiacono, G., Berta, G., Branca, C., 2000. IAA and ZR content in leek (*Allium porrum* L.), as influenced by P nutrition and arbuscular mycorrhizae, in relation to plant development. Plant Soil 226: 29-35.

Trewavas, A.J., 1981. How do plant growth substances work? Plant Cell Environ. 4: 203-228.

Trewavas, A.J., 1991. How do plant growth substances work? II. Plant Cell Environ. 14: 1-12.

Turnau, K., Kottke, I., Oberwinkler, F., 1993. Paxillus involutus-Pinus sylvestris mycorrhizae from heavily polluted forest. I. Element localization using electron energy loss spectroscopy and imaging. Bot. Acta. 106: 213-219.

Uetake, Y., Kojima, T., Ezawa, T., Saito, M., 2002. Extensive tubular vacuole system in an arbuscular mycorrhizal fungus, *Gigaspora margarita*. New Phytol. 154: 761-768.

Valcke, E., Elsen, A., Cremers, A., 1997. The use of zeolites as amendments in radiocaesium- and radiostrontium-contaminated soils: A soil-chemical approach IV. A potted soil experiment to verify laboratory-based predictions. Zeolites 18 (2-3): 225-231.

Vandenhove, H., Van Hees, M., De Brouwer, S., Vandecasteele, C.M., 1996. Transfer of radiocaesium from podzol to ryegrass as affected by AFCF concentration. Sci. Total Environ. 187: 237-245.

Vidal, M., Camps, M., Grebenshikova, N., Sanzharova, N., Ivanov, Y., Vandecasteele, C., Shand, C., Rigol, A., Firsakova, S., Fesenko, S., Levchuk, S., Cheshire, M., Sauras, T., Rauret, G., 2001. Soil- and plant-based countermeasures to reduce Cs-137 and Sr-90 uptake by

grasses in natural meadows: the REDUP project. J. Environ. Radioactiv. 56(1-2): 139-156.

Viereck, N., Hansen, P.E., Jakobsen, I., 2004. Phosphate pool dynamics in the arbuscular mycorrhizal fungus Glomus intraradices studied by in vivo ^{31}P NMR spectroscopy. New Phytol. 162: 783-794.

Voets, L., Dupré de Boulois, H., Renard, L., Strullu, D.G., Declerck, S., 2005. Development of an autotrophic culture system for the in vitro mycorrhization of potato plantlets. FEMS Microbiol. Lett. 248: 111-118.

Vosatka, M., 2001. A future role for the use of arbuscular mycorrhizal fungi in soil remediation: a chance for small-medium enterprises? Minerva Biotecnol. 13(1): 69-72.

Waegeneers, N., Camps, M., Smolders E. and Merckx, R., 2001. Genotypic effects in phytoavailability of radiocaesium are pronounced at low K intensities in soil. *Plant Soil* 235: 11-20.

Waegeneers, N., Smolders, E., Merckx, R., 2005. Modelling Cs-137 uptake in plants from undisturbed soil monoliths. J. Environ. Radioactiv. 81(2-3): 187-199.

Wang, S.G., Lin, X.G., Yin, R., Hou, Y.L., 2004. Effect of inoculation with arbuscular mycorrhizal fungi on the degradation of DEHP in soil. J. Environ. Sci. 16 (3): 458-461.

Wegner, L.H., De Boer, A.H., 1997. Properties of two outward-rectifying channels in root xylem parenchyma cells suggest a role in K^+ homeostasis and long-distance signaling. Plant Physiol. 115: 1707-1719.

Weiersbye, I.M., Straker, C.J., Przybylowicz, W.J., 1999. Micro-PIXE mapping of elemental distribution in arbuscular mycorrhizal roots of the grass, Cynodon dactylon, from gold and uranium mine tailings. Nucl. Instrum. Meth. B. 158(1-4): 335-343.

White, P.J., Bowen, H., Broadley, M., Hammond, J., Hampton, C., Payne, K., 2004. The mechanisms of caesium uptake by plants, in: Inabe, J., Tsukada, H., Takeda, A. (Eds.), Proceedings of the International Symposium on Radioecology and Environmental Dosimetry. Rokkasho, Aomori, Japan, pp. 255-262.

White, P.J., Broadley, M.R., 2000. Mechanisms of caesium uptake by plants. New Phytol. 147: 241-256.

Wirth, E., Hiersch, L., Kammerer, G., Krajewska, G., Krestel, R., Mahler, S., Römmelt, R., 1994. Transfer equations for cesium-137 for

coniferous forest understorey plant species. Sci. Total Environ. 157: 163-177.

Yoshida, S., Muramatsu, Y., 1994. Accumulation of radiocaesium in basidiomycetes collected from Japanese forests. Sci. Total Environ. 157: 197-205.

Yoshida, S., Muramatsu, Y., 1998. Concentrations of alkali and alkaline earth elements in mushrooms and plants collected in a Japanese pine forest, and their relationship with ^{137}Cs. J. Environ. Radioact. 41: 183-205.

Yuan, L., Huang, J.G., Li, X.L., Christie, P., 2004. Biological mobilization of potassium from clay minerals by ectomycorrhizal fungi and eucalypt seedling roots. Plant Soil 262(1-2): 351-361.

Zeevaart, J.A.D., Creelman, R.A., 1988. Metabolism and physiology of abscisic acid. Annu. Rev. Plant Physiol. Plant Mol. Biol. 39: 439-473.

Zhu, Y., Shaw, G., 2000. Soil contamination with radionuclides and potential remediation. Chemosphere, 41: 121-128.

Zhu, Y.G., Smolders, E., 2000. Plant uptake of radiocaesium: a review of mechanisms, regulation and application. J. Exp. Bot. 51: 1635-1645.

Zhu, Y.G., Shaw, G., Nisbet, A.F., Wilkins, B.T., 1999. Effects of external potassium supply on compartmentation and flux characteristics of radiocaesium in intact spring wheat roots. Ann. Bot. 84: 639-644.

Zhu, Y.G., Shaw, G., Nisbet, A.F., Wilkins, B.T., 2000. Effects of potassium starvation on the uptake of radiocaesium by spring wheat (*Triticum aestivum* cv. Tonic). Plant Soil 220: 27-34.

OVERVIEW OF THE SCIENTIFIC ACHIEVEMENTS

I - Scientific publications

I-I Research articles

- Declerck, S., Dupré de Boulois, H., Bivort, C., Delvaux, B., 2003. Extraradical mycelium of the arbuscular mycorrhizal fungus *Glomus lamellosum* can take up, accumulate and translocate radiocaesium under root-organ culture conditions. Environmental Microbiology 5: 510-516.

- Dupré de Boulois, H., Delvaux, B., Declerck, S., 2005. Effects of arbuscular mycorrhizal fungi on the root uptake and translocation of radiocaesium. Environmental pollution 134: 515-524.

- Voets, L., Dupré de Boulois, H., Renard, L., Strullu, D.G., Declerck, S., 2005. Development of an autotrophic culture system for the *in vitro* mycorrhization of potato plantlets. FEMS Microbiology Letters 248: 111-118.

- Dupré de Boulois, H., Voets, L., Delvaux, B., Jakobsen, I., Declerck, S., 2006. Transport of radiocaesium by arbuscular mycorrhizal fungi to *Medicago truncatula* under *in vitro* conditions. Environmental Microbiology 8(11): 1926-1934.

I-II- Review Articles

- Dupré de Boulois, H., Rufyikiri, G., Thiry, Y., Delvaux, B., Joner, E., Leyval, C., Jakobsen, I., Roos, P., Declerck, S., 2005. Use of mycorrhizal fungi for the phytostabilisation of radio-contaminated environment (European project MYRRH): Overview on the scientific achievements. Radioprotection 40: S41-S46.

- Dupré de Boulois, H., Rufyikiri, G., Thiry, Y., Delvaux, B., Joner, E.J., Leyval, C., Jakobsen, I., Roos, P., Declerck, S. Role and influence of mycorrhizal fungi on the accumulation of radionuclides by plants. In preparation, Special issue for the Journal of Environmental Radioactivity.

I-III- Book Chapters

- Rufyikiri, G., Kruyts, N., Declerck, S., Thiry, Y., Delvaux, B., Dupré de Boulois, H., Joner, E.J., 2005. Uptake, Assimilation and Translocation of Mineral Elements in Monoxenic Cultivation Systems, in: Declerck, S., Fortin, A. J., Strullu, D. G. (Eds.), In vitro culture of mycorrhizas, Springer-Verlag, Heidelberg, pp. 201-215.

- Dupré de Boulois, H., Voets, L., Declerck, S. *In vitro* compartmented systems to study transport in arbuscular

mycorrhizal symbiosis, in: Varma, A. (Ed.), Mycorrhiza Manual, second ed., Springer-Verlag, Heidelberg, accepted for publication.

II- Conference Participation

- Dupré de Boulois, H., Delvaux, B., Renard, L., Declerck, S. Uptake and translocation of radiocaesium by arbuscular mycorrhizal fungi under root-organ culture conditions. Poster presented at the 4th International Conference On Mycorrhizae, Montreal (Canada), 10-15 August 2003.

- Rufyikiri, G., Dupré de Boulois, H., Thiry, Y., Delvaux, B., Declerck, S. Role of arbuscular mycorrhizal fungi in the uptake of radionuclides by plants. Oral presentation at the 4th International Conference On Mycorrhizae, Montreal (Canada), 10-15 August 2003.

- Dupré de Boulois, H., Delvaux, B., Declerck, S. Uptake and translocation of radiocaesium by arbuscular mycorrhizal fungi. Oral presentation at the 8th Mycorrhizal Group Meeting of the Nederlands-Vlaamse Vereniging voor Ecologie, Diepenbeek (Belgium), 26 September 2003.

- Dupré de Boulois, H., Rufyikiri, G., Thiry, Y., Delvaux, B., Joner, E., Leyval, C., Jakobsen, I., Roos, P., Declerck, S. Use of mycorrhizal fungi for the phytostabilisation of radio-contaminated environment (European project MYRRH): Overview on the scientific

achievements. Oral Presentation at the International Congress ECORAD, Aix-en-Provence (France), 6-10 September 2004.

- Dupré de Boulois, H., Declerck, S. Transport of radiocaesium by arbuscular mycorrhizal fungi : from myths to reality. Poster presented at the 5th International Conference On Mycorrhiza, Granada (Spain), 23-27 July 2006.

- Dupré de Boulois, H., Voets, L., Declerck, S. From ROC to AM-P *in vitro* culture systems for arbuscular mycorrhizal fungal transport studies. Oral presentation at the 5th International Conference On Mycorrhiza, Granada (Spain), 23-27 July 2006.

- Gyuricza, V., Dupré de Boulois, H., Declerck, S. Effect of K on the transport of Cs by arbuscular mycorrhizal fungi. Abstract accepted for the 9th International Conference on the Biogeochemistry of Trace Elements, Beijing (China), 15-19 July 2007.

III- Participation in project writing

- MYCOREMED: Role of arbuscular mycorrhizal fungi on the accumulation of radiocaesium by plants. Early Stage Training (EST) project in FP6. Coordinator: Declerck, S. (UCL). Partners: White, P-J. (UK); Hedrich, R. (Germany) and Turnau, K. (Poland). Accepted, 2006-2010.

- MYCARBIO: Mycorrhizae impact on biodiversity and C-balance of grassland ecosystems under changing climate. Belspo, Science for a Sustainable Development (SSD). Coordinator: Declerck, S. (UCL). Partner: Ceulemans, R. & Nijs, I. (Universiteit Antwerpen). Accepted, 2006-2011.

IV- Training during PhD

- Risø National Laboratory, Roskilde, Denmark, 2003. "Radioisotopic labelling to measure transport of P by AM fungi". Marie Curie Host Fellowship, 4 months.

V- Teaching and student supervision

- "*In vitro* cultivation of Ri T-DNA transformed carrot roots". Class given within the "International Training on *In Vitro* Culture of Arbuscular Mycorrhizal fungi" organized by Prof. S. Declerck and Dr. S. Cranenbrouck (UCL) since 2005.

I was also appointed to prepare an international training, in collaboration with Dr. S. Cranenbrouck, on the HAM-P and AM-P *in vitro* culture systems. This work will, especially, involve the preparation of a document summarizing the techniques used to set up these systems, as well as their potentials and their limits to study AM symbioses. First training planned in June 2007.

- Supervision of three students (1 bachelor and 2 master students) who performed their theses at the laboratory of Prof. S. Declerck. Total supervision time: 42 months.

I also helped Prof. S. Declerck to supervise a junior scientist and a PhD student in their research activities (e.g. planning and designing experiments and analyses of results on Rb and Cs transport by AM fungi). Total supervision time: 6 months.

VI- Scientific collaborator and advisor

I am currently involved as scientific collaborator in the TRACEAM and FUNGIMYC projects and as scientific advisor in the MYCOREMED project.

- TRACEAM: Traceability of arbuscular mycorrhizal fungi as plant-beneficial micro-organisms in agro-environments. Early Stage Training (EST) project in FP6. Coordinator: Schuessler, A. (Germany). Partners: Declerck, S. (UCL); Martin, F. (France); Bonfante, P. (Italy). 2005-2009.
Guidance on the use of the new *in vitro* cultivation systems of AM fungi.

- FUNGIMYC: Influence of the sterol biosynthesis inhibitor fungicides on the arbuscular mycorrhizal symbiosis. Early Stage

Training (EST) project in FP6. Coordinator: Declerck, S. (UCL). Partners: Lanfranco L. (Italy); Grandmougin, A. (France). 2005-2009. Guidance on the use of the new *in vitro* cultivation systems of AM fungi & on radioisotopic labelling methods (^{33}P).

- MYCOREMED: Role of arbuscular mycorrhizal fungi on the accumulation of radiocaesium by plants. Early Stage Training (EST) project in FP6. Coordinator: Declerck, S. (UCL). Partners: White, P-J. (UK) ; Hedrich, R. (Germany) and Turnau, K. (Poland). 2006-2010. PhD student supervisor. Report coordinator.

Recent titles in the collection

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