EFFECT OF ECTOMYCORRHIZAE AND AMMONIUM ON ^{134}Cs AND ^{85}Sr UPTAKE INTO *PICEA ABIES* SEEDLINGS

Thomas K. Riesen^a & Ivano Brunner^b

^aPaul Scherrer Institute (PSI), Division for Radiation Hygiene, CH-5232 Villigen PSI, Switzerland

^bSwiss Federal Institute for Forest, Snow and Landscape Research (WSL), CH-8903 Birmensdorf, Switzerland

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Abstract

Microorganisms play an important role in the fixation of radionuclides in forest soils. In particular, fungi have the capacity to absorb and translocate radionuclides. The role of the ectomycorrhizal fungus *Hebeloma crustuliniforme* in the uptake of radiocaesium (^{134}Cs) and radiostrontium (^{85}Sr) into seedlings of Norway spruce (*Picea abies*) was investigated in a pouch test system. Inoculated and non-inoculated seedlings; seedlings inoculated during 8 and 15 weeks; seedlings exposed during 2 and 3 weeks to the radioactive solution; and seedlings grown under low and high ammonium conditions prior to the application of the radionuclides were compared. The final ^{134}Cs and ^{85}Sr activity was determined in fine-roots, main-roots, stems and needles. The results showed that ectomycorrhizae reduced the uptake of ^{134}Cs and ^{85}Sr . The degree of ectomycorrhization was of crucial importance and seemed to be governed by the period during which ectomycorrhizae were allowed to develop and by the ammonium concentration in the nutrient solution. The radionuclide uptake increased with increasing exposure time. Both radionuclides were predominantly accumulated in fine-roots. However, needles proved to describe best the result of net root uptake and translocation to the shoot. The uptake- and translocation-rates of ^{85}Sr were smaller than those of ^{134}Cs . It is assumed that the translocation is coupled with the intensity of water fluxes through the xylem and that ^{85}Sr is more readily adsorbed into mycelium or plant tissue relative to ^{134}Cs . The effect of high ammonium growth conditions was overcome by the effect of ectomycorrhization, except in needles with a very large biomass which behaved as a strong sink and led to a high accumulation of ^{134}Cs . Copyright © 1996 Elsevier Science Ltd

Keywords: Norway spruce, nitrogen, caesium, strontium, plant growth.

INTRODUCTION

After a nuclear explosion or a nuclear accident ^{137}Cs and ^{90}Sr are amongst the most significant isotopes produced in the fission of ^{235}U because of their very long half-life of 30.2 and 28.5 years, respectively (Szabo, 1993). Therefore, it is important to know their long-term

behaviour in ecosystems contaminated by them. Caesium in particular is known to be integrated very quickly into the nutrient cycle of forest ecosystems (Römmelt *et al.*, 1990). This is indicated by the fact that the major part of caesium originating from the bomb fallout of the 1950s and 1960s, or being deposited on the forest floor after the Chernobyl accident of 1986, is still detectable in the top soil layers today and that only a small part is exchangeable (Myttenaere *et al.*, 1993). High activity in fungal fruiting bodies and plants which mainly take up their nutrients from organic soil layers are another indication of this behaviour. Microorganisms and particularly fungi may retain up to 40% of deposited radiocaesium in the soil (Guillitte *et al.*, 1994; Olsen *et al.*, 1990), thereby preventing it from leaching into deeper layers (Witkamp & Barzansky, 1968; Römmelt *et al.*, 1990). Fungal mycelia have the capacity to absorb and retain or translocate radiocaesium and radiostrontium (Haselwandter & Berreck, 1994), which is shown by the high activities in fruiting bodies of ectomycorrhizae-forming basidiomycetes. Although the concentration of caesium in the soil solution is low, the soil-to-plant transfer is an important process in the long term (Myttenaere *et al.*, 1993).

In recent years, nitrogen input into forest ecosystems has increased steadily. In such ecosystems the fruiting body formation of ectomycorrhizal fungi has dropped markedly (Dighton & Jansen, 1991). Experiments have also shown that high nitrogen concentrations inhibit the growth of fungal mycelium in the soil and suppress the complete development of ectomycorrhizae (Haug *et al.*, 1992; Wallander & Nylund, 1992; Brunner & Scheidegger, 1995). The question arises as to what role ectomycorrhizae play as a link between soil solution and plant root, and to what extent a transfer of caesium and strontium into the host plant occurs (Shaw & Bell, 1994). Therefore, this study was designed to determine the uptake of radiocaesium and radiostrontium into seedlings of Norway spruce in relation to: (1) inoculation with an ectomycorrhizal fungus; (2) the time of ectomycorrhizal development; (3) the exposure time to nuclides; and (4) the ammonium concentrations in the nutrient solutions during ectomycorrhizal development.

MATERIALS AND METHODS

Growth conditions

Autoclaved polyamid/polypropylene pouches (Cellpack, Switzerland), 13 × 16 cm, longitudinally divided into two chambers with a welding seam and coated with two activated charcoal filter papers (5 × 9 cm), were moistened with 5 ml per chamber of modified Melin-Norkrans nutrient solution (MMN) without glucose (Brunner & Scheidegger, 1995). Two seeds of Norway spruce (*Picea abies* L., Karst.) were inserted into each chamber of the growth pouches and thinned to one seedling after germination. Seeds were collected from a single tree near Tägerwil (Switzerland) and were surface sterilized in 30% H₂O₂ for 20 min. After 10 weeks, four to six inoculum disks with mycelium of *Hebeloma crustuliniforme* (Bull.: St Amans) Quél. (WSL strain No. 6.2) were placed within 3 mm of the fine-roots and another 5 ml of MMN (including 5 g litre⁻¹ glucose) were added. Additional nitrogen in the form of NH₄Cl was added simultaneously with the MMN, leading to final NH₄⁺ concentrations of 7.6 mM (without additional NH₄Cl) or 44.9 mM (with additional NH₄Cl). The pH of the solutions at both NH₄⁺ concentrations was 6.1–6.2 after autoclaving and 6.5–6.8 after 2 months, irrespective of fungal inoculation (Brunner & Scheidegger, 1995). One strip of foam (1 × 1 × 3 cm) was placed beside each root to provide air space. Sterile distilled water was added as needed. Seedlings were grown in a growth chamber with a 16 h day period (PAR: 100 µmol m⁻² s⁻¹) at 20°C and 70% relative humidity. For the continuation of the trials only seedlings with more than 10 mycorrhizal root tips were used.

Application of radionuclides

Nine weeks after introducing the agar disk, ectomycorrhizae had developed on the activated charcoal filter paper. When contamination by other microorganisms occurred during the ectomycorrhizal development, the contaminated pouches were discarded. The number of replicates per trial is given below. The nutrient solution in the pouches was washed out twice with 10 ml of sterile water and afterwards 2 ml of sterile water were supplied to the bottom of the pouches (without contacting the charcoal paper) to water the plants over the main-roots during the experiment. Two days after adding sterile water the filter paper, which had started to dry, was soaked by a syringe with a solution of ¹³⁴CsCl and ⁸⁵SrCl₂ with an activity of 18 × 10³–19 × 10³ Bq ml⁻¹ each of ¹³⁴Cs and ⁸⁵Sr. The same solution was added as often as necessary to keep the filter papers wet until one day prior to the end of each experiment. In total, roots were exposed to the radioactive solution for 10 days, except in trial 3 (see below) where the exposure time was 11 or 18 days. The plants were then harvested, washed three times in a 0.1 M HCl solution to remove any ¹³⁴Cs or ⁸⁵Sr from the root surface, cleaned from adhering filter paper and mycelium by a pair of tweezers, and dissected into needles, stem, main-roots (≥ 1 mm in diameter) and fine-roots (< 1 mm in diameter). These parts

were dried separately at 100°C for 3 days, weighed and dissolved in 2.5 ml 65% HNO₃ and 1.5 ml 30% H₂O₂ in a high pressure microwave (Milestone MLS 1200 Mega, Microwave Laboratory Systems) for 10 min at 250 W, 6 min at 500 W, 2 min at 0 W, 6 min at 450 W and 5 min of ventilation. ¹³⁴Cs and ⁸⁵Sr activities were measured in a gamma spectrometer using a Ge-detector connected to a multichannel analyser.

Treatments

Four trials were established using the following method:

1. Effect of mycorrhizal inoculation:
Comparison between non-inoculated (7 replicates) and infected (9 replicates) seedlings incubated during 8 weeks and grown under low ammonium conditions.
2. Effect of degree of mycorrhization:
Comparison between seedlings inoculated during 8 (9 replicates) and 15 weeks (14 replicates) grown under low ammonium conditions.
3. Effect of duration of labelling:
Comparison between inoculated seedlings exposed during 11 or 18 days to the active solution (8 replicates each).
4. Effect of interaction of competing NH₄ ions:
Comparison between low and high ammonium growth conditions in trials 1 and 2. The number of replicates for the high ammonium growth conditions are 5 (non-inoculated) and 7 (infected) for trials 1 and 7 (8 weeks formation time) and 8 (15 weeks formation time) for trial 2.

All relevant data were subjected to a two-factor, non-repeated measures, unbalanced model; unequal sample size, analysis of variance (StatView II, Abacus concepts) to test for fungal treatment effects and the influence of ammonium nutrition.

RESULTS

All results of the activity measurements were related to the dry wt of the corresponding plant parts throughout and listed as specific activities or tissue concentrations (Bq mg⁻¹ dry wt). This interpretation of results was given preference to figures of total uptake (Bq) and allocation (Bq in each plant part), since the investigations focused more on radionuclide uptake under different environmental conditions than on allocation.

Ectomycorrhization

Tables 1–3 show the effect of ectomycorrhization on the dry wt and on radionuclide accumulation in spruce seedlings grown under low and high ammonium conditions before being exposed to a radioactive solution. Needles, stems and roots of ectomycorrhizal plants have

a higher biomass after 8 weeks compared to non-ectomycorrhizal controls. The shoot/root ratio in non-ectomycorrhizal and ectomycorrhizal plants is 2.2 and 1.7, respectively, which points to an increase of the total root dry wt (plant and fungus biomass) in ectomycorrhizal plants (data not shown). Contrary to the dry wt, the ^{134}Cs and ^{85}Sr activity is generally not significantly different in ectomycorrhizal plants. The pattern of distribution within the plant is similar for both nuclides. However, ^{85}Sr uptake, accumulation and translocation was two–three times lower in roots and stems and very low in needles compared to ^{134}Cs . Independent of the stage of ectomycorrhization, both nuclides are primarily accumulated in roots, mainly in fine-roots. This distribution is also expressed by the small shoot/root ratio of the ^{134}Cs and ^{85}Sr activities in ectomycorrhizal and non-ectomycorrhizal seedlings (data not shown). In needles and stems a similar ^{134}Cs activity is measured, while the ^{85}Sr activity is much lower in needles compared to stems. This difference for ^{85}Sr becomes even more pronounced in ectomycorrhizal seedlings. The $^{134}\text{Cs}/^{85}\text{Sr}$ ratio of fine-roots between ectomycorrhizal and non-ectomycorrhizal seedlings, is 2.6 and 2.5, respectively (data not shown). The same ratio for needles is 12.1 and 27.0, respectively (data not shown).

Developing time of ectomycorrhizae

Tables 4–6 show the effect of the formation time of ectomycorrhizae and seedling age on seedling dry wt

and ^{134}Cs and ^{85}Sr uptake. The total dry wt of seedlings which were allowed to form ectomycorrhizae during 15 weeks from inoculation was significantly higher compared to seedlings with 8 weeks formation time (Table 4). The weight increase of needles, stems, main-roots and fine-roots from 8 to 15 weeks after ectomycorrhizal developing time was 1.5, 1.2, 1.3 and 3.1, respectively. Three–ten times less ^{134}Cs and ^{85}Sr were accumulated in seedlings with 15 weeks ectomycorrhizal formation time compared to those with 8 weeks of formation time (Tables 5 and 6). The differences between 8 and 15 weeks formation time are statistically significant for both nuclides and all plant parts.

Exposure time

The comparison between 11 and 18 days of exposure to a solution of $^{134}\text{CsCl}$ and $^{85}\text{SrCl}_2$ showed a clear time dependence of radionuclide uptake (Table 7). Significantly more ^{134}Cs and ^{85}Sr were accumulated in all plant parts after 18 days than after 11 days. The high values in stems with 11 days of exposure result from one single sample which lays far outside the range of the other values. The two-fold increase of both nuclides at steady plant dry wt points to a flux of dissolved radionuclides throughout the xylem due to the transpiration of the plant.

Ammonium concentration in the nutrient solution

Tables 1–6 compare the effect of low and high ammonium concentrations of nutrition solution on seedling

Table 1. Dry wt (mg) of *Picea abies* seedlings, as influenced by ectomycorrhizal fungi and ammonium concentration in the growth medium (Low N, High N). Seedlings were incubated during 8 weeks prior to exposure to ^{134}Cs and ^{85}Sr for 10 days

Ectomycorrhizal fungi	Needles		Stem		Main-roots		Fine-roots		Total	
	Low N ^a	High N ^b	Low N	High N	Low N	High N	Low N	High N	Low N	High N
Non-inoculated	30.1 A ^c	16.6 B	3.4 A	3.4 A	11.3 A	3.8 B	3.7 A	3.2 A	48.6 ^d A	27.0 ^f B
Infected	35.9 A	58.1 A	4.2 A	4.6 A	17.6 A	18.0 A	5.9 A	6.1 A	63.6 ^e A	86.9 ^g A
Probability										
Fungus (F)	0.0059		ns ^h		0.0007		0.0201		0.0019	
N treatment	ns		ns		ns		ns		ns	
F × N	0.0312		ns		ns		ns		ns	

^aLow N = 7.6 mM. ^bHigh N = 44.9 mM. ^cDifferent letters within columns indicate a significant difference ($p \leq 0.05$). ^dn = 7. ^en = 9. ^fn = 5. ^gn = 7. ^hns = not significant.

Table 2. Uptake and translocation of ^{134}Cs (Bq mg⁻¹) in seedlings of *Picea abies*, as influenced by ectomycorrhizal fungi and ammonium concentration in the growth medium (Low N, High N). Seedlings were incubated during 8 weeks prior to exposure to ^{134}Cs for 10 days

Ectomycorrhizal fungi	Needles		Stem		Main-roots		Fine-roots		Total	
	Low N ^a	High N ^b	Low N	High N	Low N	High N	Low N	High N	Low N	High N
non-inoculated	83.7 A ^c	52.2 A	81.8 A	41.4 A	131.5 A	115.6 A	567.3 A	102.3 B	117.0 ^d A	62.7 ^f B
Infected	45.9 B	75.2 A	50.3 B	45.3 A	135.6 A	133.5 A	331.9 A	234.4 A	93.8 ^e A	95.1 ^g A
Probability										
Fungus (F)	ns ^h		ns		ns		ns		ns	
N treatment	ns		0.0435		ns		0.0062		0.0118	
F × N	0.0028		ns		ns		ns		0.0086	

^aLow N = 7.6 mM. ^bHigh N = 44.9 mM. ^cDifferent letters within columns indicate a significant difference ($p \leq 0.05$). ^dn = 7. ^en = 9. ^fn = 5. ^gn = 7. ^hns = not significant.

dry wt and radionuclide uptake in non-ectomycorrhizal and ectomycorrhizal seedlings, and in seedlings allowed to develop ectomycorrhizae during 8 and 15 weeks. High ammonium growth conditions inhibit ectomycorrhizae-free seedling development compared to low ammonium conditions, which becomes most apparent in the dry wt of needles (Table 1). In combination with ectomycorrhizae, high ammonium growth conditions promote seedling growth which is again most clearly expressed in the needle dry wt. As indicated by the analysis of variance, ectomycorrhization influences the dry wt of the different plant parts much more than a high ammonium concentration in the growth medium, except in needles where both parameters contribute to an enhanced needle growth. A similar result is obtained when comparing the dry wt of ectomycorrhizal seedlings which were grown for 8 and 15 weeks under low and high ammonium conditions (Table 4). The formation time for ectomycorrhizae imposes a greater effect on the dry wt than the ammonium concentration. A combination of both parameters leads to a significant increase in the dry wt of needles only.

Caesium uptake in non-ectomycorrhizal seedlings grown under low ammonium conditions is higher compared to seedlings grown under ammonium-rich conditions (Table 2). This is most evident in fine-roots. In needles, the decrease of ^{134}Cs activity due to ectomycorrhization is offset by the effect of high ammonium growth conditions prior to exposure to the active solution, as seen before by comparing the dry wt. The analysis

of variance indicates that the ammonium concentration during seedling growth is a more important factor in ^{134}Cs uptake than the degree of ectomycorrhization (ectomycorrhizal versus non-ectomycorrhizal). When comparing ^{134}Cs uptake in seedlings grown for 15 weeks under ammonium-rich and ammonium-poor conditions prior to the exposure to the radionuclide solution, an increase in ^{134}Cs uptake or accumulation was observed, except in stems (Table 5). This result contradicts that obtained for 8-week-old seedlings. The analysis of variance indicates a significant interaction between ammonium treatment and ectomycorrhizae formation time in needles only. However, there is a tendency for high ammonium growth conditions to diminish the differences in ^{134}Cs uptake during the two formation periods.

Strontium uptake in non-ectomycorrhizal seedlings grown under low ammonium conditions follows no clear pattern compared to seedlings grown under ammonium-rich conditions (Table 3). Only in fine-roots do high ammonium growth conditions reduce the ^{85}Sr activity significantly. In needles, stems and main-roots of ectomycorrhizal plants, the effect of ectomycorrhization is offset by the high ammonium growth conditions prior to exposure to the active solution. In fine-roots of ectomycorrhizal plants, ammonium-rich growth conditions reduce ^{85}Sr uptake, unlike the case for other plant parts. Comparing the ^{85}Sr uptake in seedlings grown for 8 or 15 weeks prior to the exposure to the radionuclide solution, no difference was observed concerning the effects of ammonium nutrition (Table 6). The much

Table 3. Uptake and translocation of ^{85}Sr (Bq mg^{-1}) in seedlings of *Picea abies*, as influenced by ectomycorrhizal fungi and ammonium concentration in the growth medium (Low N, High N). Seedlings were incubated during 8 weeks prior to exposure to ^{85}Sr for 10 days

Ectomycorrhizal fungi	Needles		Stem		Main-roots		Fine-roots		Total	
	Low N ^a	High N ^b	Low N	High N	Low N	High N	Low N	High N	Low N	High N
Non-inoculated	6.9 A ^c	10.1 A	52.3 A	33.8 A	102.5 A	124.0 A	221.9 A	96.1 A	45.5 ^d A	32.1 ^f A
Infected	1.7 B	9.7 A	23.0 A	40.9 A	69.1 B	93.7 A	134.5 B	91.5 A	32.6 ^e A	35.1 ^g A
Probability										
Fungus (F)	ns ^h		ns		ns		ns		ns	
N treatment	ns		ns		ns		0.0051		ns	
F × N	ns		ns		ns		ns		ns	

^aLow N = 7.6 mM. ^bHigh N = 44.9 mM. ^cDifferent letters within columns indicate a significant difference ($p \leq 0.05$). ^dn = 7. ^en = 9. ^fn = 5. ^gn = 7. ^hns = not significant.

Table 4. Dry wt (mg) of *Picea abies* seedlings, as influenced by ectomycorrhizal formation time (8 and 15 weeks) and ammonium concentration in the growth medium (Low N, High N). Exposure to ^{134}Cs and ^{85}Sr was 10 days.

Weeks	Needles		Stem		Main-roots		Fine-roots		Total	
	Low N ^a	High N ^b	Low N	High N	Low N	High N	Low N	High N	Low N	High N
8	35.9 B ^c	58.1 B	4.2 A	4.6 B	17.6 A	18.0 A	5.9 B	6.1 B	63.5 ^d B	86.9 ^f B
15	52.2 A	127.4 A	5.2 A	7.9 A	23.7 A	29.1 A	18.4 A	22.9 A	99.5 ^e A	187.3 ^g A
Probability										
Weeks (W)	0.0002		0.0052		0.0259		0.0001		0.0002	
N treatment	0.0001		0.0441		ns		ns		0.0018	
W × N	0.0161		ns ^h		ns		ns		ns	

^aLow N = 7.6 mM. ^bHigh N = 44.9 mM. ^cDifferent letters within columns indicate a significant difference ($p \leq 0.05$). ^dn = 9. ^en = 14. ^fn = 7. ^gn = 8. ^hns = not significant.

lower uptake of ^{85}Sr in 15-week-old seedlings is due to the extended formation time of ectomycorrhizae. However, in needles and stems the relative ^{85}Sr uptake was significantly increased by ammonium treatment (cf. ANOVA, Table 6).

DISCUSSION

Our results clearly show that ectomycorrhization reduces the net uptake of radiocaesium and radiostrontium in spruce seedlings grown in a pouch test system. The same effect was previously reported after a short-term trial with heather (*Calluna vulgaris* L.) (Clint & Dighton, 1992). These results contradict our assumption that ectomycorrhizal plants would show an enhanced radionuclide uptake, as deduced from the generally accepted finding that mycorrhizae have a positive effect on the mineral nutrition of plants. This view was supported by reports which found that ectomycorrhizal infection leads to an enhanced radionuclide uptake: ectomycorrhizal *Pinus ponderosa*, Dougl. ex Laws., and *P. radiata*, D. Don, seedlings were able to remove three–five times more ^{85}Sr from contaminated soil than seedlings without ectomycorrhizae (Entry *et al.*, 1994). In another trial, ectomycorrhizae significantly increased ^{86}Rb uptake rates, but decreased the amount of ^{86}Rb released to the external solution (Rygiewicz *et al.*, 1984). In addition to this, some VAM (Vesicular Arbuscular Ectomycorrhizae) are described as enhancing the uptake

of radiocaesium in *Paspalum notatum* (Haselwandter *et al.*, 1994). However, VAM can also decrease radiocaesium content in *Festuca ovina* (Haselwandter & Berreck, 1994). Obviously, results concerning radionuclide uptake remain controversial and are still inadequate for drawing any general conclusion (Haselwandter & Berreck, 1994).

Radiocaesium and radiostrontium are predominantly accumulated in roots of both ectomycorrhizal and non-ectomycorrhizal seedlings. A distinction of the activity distribution between fungus and root tissue is not possible with the design of our experiment. However, it is known that elements absorbed from the soil solution are either first accumulated in the fungal sheath, which seems to act as a storage organ (Alexander, 1983), or form a pool with a slow turnover rate (Clint *et al.*, 1991) and are mobilized later on (Harley, 1978). Investigations on the uptake and localization of ^{134}Cs in Norway spruce seedlings as influenced by ectomycorrhizae and caesium/potassium ratio (Brunner *et al.*, 1996) confirm these findings. Flux studies on common grassland fungi indicate that more than 40% of the caesium taken up is bound within the hyphae (Dighton *et al.*, 1991). Radionuclide uptake rates and storage sites are additionally affected by the specific tree–ectomycorrhizae association (Entry *et al.*, 1994).

The results of our experiment with ectomycorrhizae allowed to develop for 8 and 15 weeks indicate that the uptake and consequent translocation of radionuclides considerably decreases in seedlings infected 15 weeks

Table 5. Uptake and translocation of ^{134}Cs (Bq mg^{-1}) in *Picea abies* seedlings, as influenced by ectomycorrhizal formation time (8 and 15 weeks) and ammonium concentration in the growth medium (Low N, High N). Exposure to ^{134}Cs was 10 days

Weeks	Needles		Stem		Main-roots		Fine-roots		Total	
	Low N ^a	High N ^b	Low N	High N	Low N	High N	Low N	High N	Low N	High N
8	45.9 A ^c	75.2 A	50.3 A	45.3 A	135.6 A	133.5 A	331.9 A	234.4 A	93.8 ^d A	95.1 ^f A
15	8.4 B	17.2 B	14.1 B	13.2 B	22.2 B	42.6 B	69.5 B	101.7 B	22.3 ^e B	31.0 ^g B
Probability										
Weeks (W)	0.0001		0.0001		0.0001		0.0001		0.0002	
N treatment	0.0001		ns ^h		ns		ns		ns	
W × N	0.0121		ns		ns		ns		ns	

^aLow N = 7.6 mM. ^bHigh N = 44.9 mM. ^cDifferent letters within columns indicate a significant difference ($p \leq 0.05$). ^dn = 9. ^en = 14. ^fn = 7. ^gn = 8. ^hns = not significant.

Table 6. Uptake and translocation of ^{85}Sr (Bq mg^{-1}) in *Picea abies* seedlings, as influenced by ectomycorrhizal formation time (8 and 15 weeks) and ammonium concentration in the growth medium (Low N, High N). Exposure to ^{85}Sr was 10 days

Weeks	Needles		Stem		Main-roots		Fine-roots		Total	
	Low N ^a	High N ^b	Low N	High N	Low N	High N	Low N	High N	Low N	High N
8	1.7 A ^c	9.7 A	23.0 A	40.9 A	69.1 A	93.7 A	134.5 A	91.5 A	32.6 ^d A	35.1 ^f A
15	0.5 A	4.7 A	4.5 B	26.6 A	11.6 B	19.7 B	13.1 B	13.7 B	5.5 ^e B	8.9 ^g B
Probability										
Weeks (W)	0.027		0.0346		0.0001		0.0001		0.0001	
N treatment	0.0001		0.0112		ns		ns		ns	
W × N	ns ^h		ns		ns		ns		ns	

^aLow N = 7.6 mM. ^bHigh N = 44.9 mM. ^cDifferent letters within columns indicate a significant difference ($p \leq 0.05$). ^dn = 9. ^en = 14. ^fn = 7. ^gn = 8. ^hns = not significant.

prior to the exposure to radionuclides. The total dry wt of ectomycorrhizal fine-roots increases by a factor of two in relation to the other plant parts, which points to morphological changes leading to a reduced radionuclide uptake. It is assumed that the fungal mantle, caused by a higher degree of fungal infection, acts as a barrier, which causes an increase in the thickness of the fungal sheath or a lignification of the roots, thereby inhibiting the uptake of ^{134}Cs and ^{85}Sr . Possible changes in seedling physiology may be another reason for reduced root uptake, since rapid growth and bud formation during the first 2 months tend to increase the nutrient requirements of seedlings. These requirements are reduced during the second two months, which coincide with the end of the first vegetation period in nature. Another parameter not considered because it cannot be quantitatively measured, is the uptake of radionuclides through the non-mycorrhizal part of the root or by the extra-matricular mycelium. Contrary to root uptake, the distribution and translocation rate of ^{134}Cs and ^{85}Sr does not seem to be affected by morphological and physiological changes already observed in seedlings with more developed ectomycorrhizae at this growth stage. Although the dry wt of fine-roots increases significantly in relation to the other plant parts (Table 4), the mean activity per dry wt of both nuclides is reduced in the other plant parts by about the same range of magnitude as in the fine-roots (Tables 5 and 6). The same observation holds true for ammonium-rich growth conditions. Apart from roots, needle activity proved to be a most useful indicator of net root uptake and ensuing translocation from roots to shoot for ^{134}Cs and ^{85}Sr . At this growth stage of the seedling, the needles are not in direct contact with the radionuclide solution and behave as a sink for nutrient uptake. Despite the low activity in needles compared to roots, not only do the results of different treatments and changes in the degree of ectomycorrhization become obvious, but also the different behaviour of ^{134}Cs and ^{85}Sr during uptake and translocation is highlighted. Since phloem fluxes from older to younger needles, as occurs in several year old plants (Ertel & Ziegler, 1991), cannot take place in our trial and since the needles have never been in direct contact with the active solution, translocation from roots to shoot occurs exclusively through the xylem. Thus, transpirational water loss may be the main cause for translocation of the radionuclides. The hypothesis exists that

the radionuclide concentration is directly related to the intensity of the water flux into the plant. Our result of a two-fold increase of both nuclides at steady plant dry wt within a week supports this assumption. Entry *et al.* (1993) found the total accumulation of ^{134}Cs to be curvilinearly related to the duration of exposure to the radionuclide solution. In addition to this, the physical properties of the radionuclides and the mechanism of their uptake by plants play an important role. Caesium net uptake is markedly higher than that of strontium. Bell *et al.* (1988) cite a similar result from experiments with plants grown in culture solutions. An opposing result may be obtained if seedlings are grown in soil (Bell *et al.*, 1988).

As with the uptake rate, the translocation rates of ^{134}Cs and ^{85}Sr differ, as shown by comparing the $^{134}\text{Cs}/^{85}\text{Sr}$ ratio in roots and needles of ectomycorrhizal and non-ectomycorrhizal seedlings. If no discrimination occurs between the two nuclides, the ratio should be similar in fine-roots and in needles. This was not the case in our trial, where the ratio in needles was higher than in fine-roots: 12.1 versus 2.6 in ectomycorrhizal and 27.0 versus 2.5 in non-ectomycorrhizal seedlings. This means that considerably more ^{134}Cs than ^{85}Sr was translocated from fine roots to needles and accumulated there, probably being strongly adsorbed at the fungal mantle and at plant cells. This assumption is supported by our findings that in ectomycorrhizal seedlings the translocation rate is less than in non-ectomycorrhizal seedlings. Hagemeyer and Lohrie (unpublished data) investigated the distribution of Cd and Zn in the xylem rings of young Norway spruce trees and concluded that the incorporation of trace elements seems to be a function of water transport capacity and of the number of binding sites for Cd^{2+} and Zn^{2+} ions in xylem vessel walls.

The results obtained with our pouch test system are difficult to relate to the many investigations of the influence of raised ammonium conditions on ^{134}Cs uptake. When the radioactive solution is added to the charcoal filter paper, the nutrient solution is replaced by sterile water, the ectomycorrhizae are established, and the seedlings are conditioned by the preceding nutrient regime. Differences in the uptake rate of ^{134}Cs and ^{85}Sr in the seedlings are, therefore, mainly a consequence of structural changes in the root area and the amount of fungal biomass developed. This could be demonstrated

Table 7. Dry wt and uptake and translocation of ^{134}Cs and ^{85}Sr into ectomycorrhizal seedlings of *Picea abies*, as influenced by the exposure time to ^{134}Cs and ^{85}Sr . Seedlings were incubated during 8 weeks after fungal inoculation, prior to the exposure to the radionuclides

	Exposure (days)	Needles	Stem	Main-roots	Fine-roots	Total
Dry wt (mg)	11	32.5 A ^a	3.3 A	12.5 A	12.3 A	60.5 A
	18	28.5 A	3.0 A	13.8 A	9.6 A	54.9 A
^{134}Cs (Bq mg ⁻¹)	11	6.6 B	33.2 A	25.3 B	46.9 B	18.4 B
	18	11.4 A	16.9 A	53.8 A	91.6 A	37.3 A
^{85}Sr (Bq mg ⁻¹)	11	0.3 B	7.5 A	16.4 A	15.2 B	6.7 B
	18	1.1 A	7.9 A	31.7 A	32.8 A	15.0 A

^aDifferent letters within columns indicate a significant difference ($p \leq 0.05$, $n = 8$).

by Brunner and Scheidegger (1995), who showed that at the same ammonium concentrations as were used in this trial, the number of mycorrhizas did not change but the Hartig net (the interface between root tissue and mycelium) was not developed in roots grown under rich ammonium conditions. It is known that the addition of salts to nutrient solutions alters the metabolic processes with reference to ion accumulation and transport, and also changes the root number and absorbing surfaces (Jackson *et al.*, 1965). An excess of ammonium in the nutrient medium may also lead to a decline of the root mass (Boxman & Roelofs, 1988; Van Dijk *et al.*, 1990; Olsthoorn *et al.*, 1991) and a decrease in the fungal biomass (Alexander, 1983). In general, the number of symbiotic infections (Boxman & Roelofs, 1988) and the number of fine roots decrease along with the development of extra-matricular mycelium (Pearson & Stewart, 1993). Therefore, radionuclide uptake of ectomycorrhizal and non-ectomycorrhizal fine-roots grown in an ammonium-rich solution should be lower than in seedlings grown in an ammonium-poor solution. This assumption was confirmed for non-ectomycorrhizal seedlings in our trial (Tables 1–3). The ammonium treatment reduced the seedling biomass, especially of needles, which resulted in a reduced radionuclide uptake. In infected seedlings, ectomycorrhizae compensated for this reduction and the biomass of needles increased, contrary to the former case. This interrelationship between the ammonium treatment and ectomycorrhization makes it difficult to accurately assess their influence on the final radionuclide concentration in the seedlings. It is hypothesized that the high increase of biomass due to the ammonium-rich nutrition leads to an enhanced transpiration rate, an enhanced xylem flux and, therefore, an enhanced radionuclide uptake. This effect is masked by the effect of ectomycorrhization which reduces radionuclide uptake, as seen previously. The ectomycorrhization itself is governed by the ammonium concentration in the nutrient medium. Differences between treatments in fine-roots of young seedlings are for the most part caused by the ammonium concentration rather than by the ectomycorrhization, according to the ANOVA in Tables 2 and 3. A further ectomycorrhization, as occurs in older seedlings, confirms our assumption that ectomycorrhizae act as a barrier to radionuclide uptake (Tables 5 and 6). The effect of the ammonium treatment is definitely overcome by the effect of ectomycorrhization, except in needles with a very large biomass which acts as a strong sink.

To conclude, it is suggested that ^{134}Cs and ^{85}Sr are translocated within young spruce seedlings in the xylem. More ^{85}Sr is absorbed than ^{134}Cs , which explains their different pattern of distribution within the plant. The uptake rate of ^{134}Cs and ^{85}Sr is determined by the degree of ectomycorrhization, the biomass of needles and, therefore, the rate of metabolism of the seedling. ^{134}Cs is taken up more easily than ^{85}Sr . The degree of ectomycorrhization depends on the amount of time ectomycorrhizae are allowed to

develop and on the ammonium concentration in the nutrient solution. Needles proved to be a valuable indicator in describing the final result of ^{134}Cs and ^{85}Sr uptake by roots.

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