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ORIGINAL PAPER

Water transfer via ectomycorrhizal fungal hyphae to conifer seedlings

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Abstract Little is known about water transfer via mycorrhizal hyphae to plants, despite its potential importance in seedling establishment and plant community development, especially in arid environments. Therefore, this process was investigated in the study reported in this paper in laboratory-based tripartite mesocosms containing the shrub *Arctostaphylos viscida* (manzanita) and young seedlings of sugar pine (*Pinus lambertiana*) and Douglas-fir (*Pseudotsuga menziesii*). The objectives were to determine whether water could be transported through mycorrhizal symbionts shared by establishing conifers and *A. viscida* and to compare the results obtained using two tracers: the stable isotope deuterium and the dye lucifer yellow carbohydrazide. Water containing the tracers was added to the central compartment containing single manzanita shrubs. The fungal hyphae were then collected as well as plant roots from coniferous seedlings in the other two compartments to determine whether water was transferred via fungal hyphae. In addition, the length of the hyphae and degree of mycorrhizal colonisation were determined. Internal transcribed spacer–restriction fragment length polymorphism (ITS-RFLP) analysis was used to identify the fungal species involved in dye (water) transfer. Results of the stable isotope analysis showed that water is transferred via mycorrhizal hyphae, but isotopically labelled water was only detected in Douglas-fir roots, not in sugar pine roots. In contrast, the fluorescent dye was transported via mycorrhizal hyphae to both Douglas-fir and sugar pine seedlings. Only 1 of 15 fungal morphotypes (identified as *Atheliaceae*) growing in the mesocosms transferred the dye. Differences were detected in the water transfer patterns indicated by the deuterium and fluorescent dye tracers, suggesting that the two labels are transported by different mechanisms in the same hyphae and/or that different fungal taxa transfer them via different routes to host plants. We conclude that both tracers can provide information on resource transfer between fungi and plants, but we cannot be sure that the dye transfer data provide accurate indications of water transfer rates and patterns. The isotopic tracer provides more direct indications of water movement and is therefore more suitable than the dye for studying water relations of plants and their associated mycorrhizal fungi.

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Introduction

Mycorrhizal fungi play a fundamental role in the uptake and transport of nutrients between the bulk soil and plant roots (Smith and Read 1997). There is also some evidence that they contribute to water acquisition in plant rhizospheres (Duddridge et al. 1980). However, the extent to which water is transported through mycorrhizal fungi to their host plants is still poorly understood. Water uptake and transport capacity between mycorrhizal fungi and host plants may be related to the capacity of different ectomycorrhizal (ECM) fungi to produce rhizomorphs, and the rhizomorphs produced by different ECM species may differ both structurally and functionally (Agerer 2001). Such differences may affect the capacity of different fungi to transport and deliver water to recipient plants. Therefore, one research challenge in mycorrhizal biology is finding suitable methods to investigate the movement and importance of water uptake and exchange between soils, fungi and plants.

The development of tracer techniques using isotopes and dyes has provided valuable methods for detecting the exchange of water and other resources between woody plants (mainly trees) and their rhizospheres. For example, Duddridge et al. (1980) successfully used $^{3}H_2O$ to investigate water movement through hyphae to pine seedlings, and more recently, Querejeta et al. (2003) used a fluorescent dye to detect and trace water transfer from oak (*Quercus*) roots directly to their mycorrhizal symbionts (i.e. the reverse transfer from that documented by Duddridge et al. 1980) during severe soil drying episodes. In these kinds of investigations, it is assumed that ECM fungi, with their extensive rhizomorphs, provide a low-resistance, and perhaps preferred, pathway for water transport, and such transport can occur over ecologically significant distances to recipient plants (Duddridge et al. 1980; Brownlee et al. 1983; Lamhamedi et al. 1992).

In this study, we examined the potential for water transport between mycorrhizal fungi and conifer seedlings (sugar pine and Douglas-fir) that co-occur with manzanita shrubs in a drought-prone, mid-elevation plant community in California's Sierra Nevada Mountains. These species inhabit sites where water is a critical limiting resource and, therefore, may determine seedling establishment within the Mediterranean climate zone they occupy. Moreover, it has been proposed that water competition between the establishing conifer seedlings and the well-established shrubs in this community may adversely affect conifer regeneration (Anderson and Helms 1994; Royce and Barbour 2001). For this reason, Sierran forest managers often remove shrubs, including *Arctostaphylos* (manzanita) species, before attempting to establish a new tree stand. In contrast to this view, field surveys conducted at coastal chaparral sites suggest that the conifer *Pseudotsuga menziesii* does not establish in chaparral vegetation dominated by *Adenostoma fasciculatum* unless the stand is occupied by *Arctostaphylos glandulosa* and/or *A. montana* [currently named *A. hookeri* subsp. *Montana* (Parker et al. 2007); Dunne and Parker 1999]. Furthermore, Horton et al. (1999) found no significant differences in soil surface temperatures, light levels or allelopathic inhibition between areas dominated by the different shrub species and suggested that the ECM fungi associated with *Arctostaphylos* shrubs can contribute to the establishment of *P. menziesii* seedlings. It has also been observed that conifers are establishing to a greater extent in *Arctostaphylos* shrub patches in the Sierra Nevada mountain range of California than in patches dominated by other species. To resolve these conflicting observations and practices, we identified a need to establish whether or not ECM fungi can transfer water to tree seedlings and, if so, the nature of the mechanisms involved using two independent (dye and isotope) tracers. If ECM fungi facilitate water acquisition or are shared between conifers and common shrubs such as *Arbutus* or *Arctostaphylos*, they may play a major role in plant community development (Molina and Trappe 1982; Perry et al. 1989).

Using laboratory-based microcosms, we applied both stable isotope and dye tracers to examine the interactions between tree seedlings and shrubs that share mycorrhizal symbionts. Our goals were (1) to determine if water could be transported through mycorrhizal symbionts that establishing conifers and *Arctostaphylos viscida* share and (2) to compare (for the first time) results obtained using the two different kinds of tracers. If water is acquired and shared, this may help explain field observations of conifer seedling establishment under "nurse" manzanita shrubs and the apparently conflicting but common management practices that favour shrub removal.

Materials and methods

The experiments were carried out in specially designed open-topped mesocosms (Fig. 1a and b) with three compartments: a central compartment and two side compartments with dimensions of 30×15×5 cm and 15×5×5 cm, respectively, containing a growth medium of quartz sand (particle size range, 0.15–0.5 mm) with a bulk density of 1.4 kg dm^{-3}. These compartments were housed in a container made of plexiglass in the following manner. One of the long sides and the bottom were both made of 1/4″

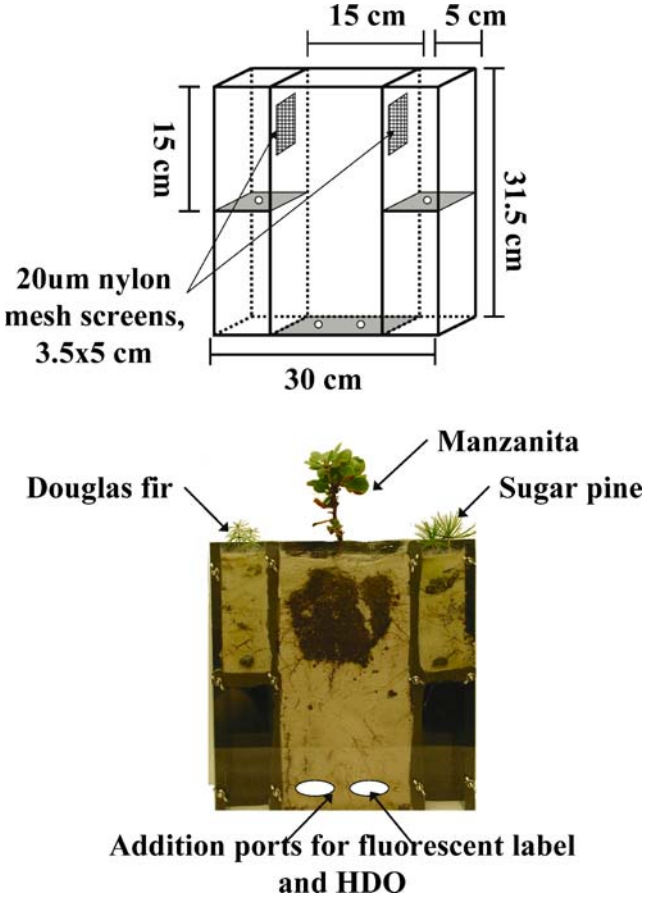

Fig. 1 Diagram (**a**) and photograph (**b**) of one of the mesocosms. The sizes of the different compartments and location of the root barriers are shown in **a**. Positions of the ports where the dye and isotope labels were injected are shown in **b**

thick black plexiglass, the other long side was a clear viewing window (Fig. 1b) made of 1/4″ thick plexiglass, and all of the other walls were made of 1/2″ thick black plexiglass. The compartments were connected by 5×3.5 cm holes covered by 20 μm nylon mesh screens (magna nylon membrane, GE Osmonics), held in place around their perimeters by 1/8″ thick clear Plexiglass glued to the compartment walls. The nylon screens were intended to prevent the growth of roots, but not mycorrhizal hyphae, between compartments. Holes (1/2 cm in diameter) drilled in the bottom panels of the central compartments were covered with the nylon mesh to prevent roots growing through them. All of the walls were glued together except the clear viewing window, which was mounted with wing nuts so it could be opened. The connections between the clear viewing window and all the other walls were isolated with waterproof weather strips made of airtight-closed cell vinyl foam (supplied by Dennis W J). In the lower part of the clear viewing window, there were two injection ports (1/2 cm in diameter) that were covered with waterproof putty (Terostat II, Henkel Teroson GmbH, Heidelberg, Germany).

Plant establishment in the mesocosms

Three-year-old manzanita (*Arctostaphylos viscida*) shrubs were transplanted from the field into the central compartments of the mesocosms in April 2001. Douglas-fir (*P. menziesii*) and sugar pine (*Pinus lambertiana*) seeds were germinated on wet tissue paper and transferred to the left and right compartments of the mesocosms, respectively, after 5–12 days in mid-May 2001 (Fig. 1b). The pots were irrigated three times every week with deionised water to near-field capacity until the drought treatment was initiated in January 2003. All of the compartments in the mesocosms were irrigated with a modified Hoaglands solution and an NH_4Cl-solution once a week until the end of August 2002 as detailed in Table 1. Growth chamber temperatures were maintained at 16/25°C in 14:10 h night/day cycles, and relative humidity varied between 30–60%. The seedlings were inoculated with mycorrhizal fungi twice during the course of the experiment. On the first occasion, in June 2001, fresh conifer and manzanita root fragments including colonised mycorrhizal roots were placed on top of the fine roots belonging to all three plant species, and on the second occasion, three months later, untreated field soil containing fungal spores were added to each compartment. The root segments and soil were collected from a mixed-conifer stand with patches of manzanita shrubs at the Teakettle Experimental Forest, ca. 80 km east of Fresno, California, in the north drainage of the Kings River (36°58′N, 119°03′W, 2,200 m elevation). The mycorrhizal inoculation was repeated because colonisation was found to be very poor 3 months after the first inoculation. Hyphal connections between the compartments took 14–18 months to become established to the extent where colonised root tips and abundant fungal mycelium were easily visible.

Table 1 Amount of nutrients added per week to each plant

Element	Amount of nutrients per plant and week
N	0.51 mg
K	0.49 mg
Mg	0.10 mg
P	0.06 mg
Ca	0.40 mg
S	0.13 mg
Fe	0.01 mg
Na	1.02 mg
Cl	1.07 mg
B	1.02 μg
Mn	1.01 μg
Zn	0.11 μg
Cu	0.03 μg
Mo	0.02 μg

Experimental treatments

The drought treatment was initiated by withholding water. The water content in the mesocosms was monitored by regularly weighing them, and when the volumetric soil water content of the entire mesocosms had fallen to approximately 5%, 40 ml of isotopically labelled water (D, 49.9 at.%; IsoTech, New Jersey, USA) containing lucifer yellow carbohydrazide (LYCH; 0.01% *w/v*, MW 457, Molecular Probes, Oregon, USA) was injected through the injection ports at the base of the central (manzanita) compartment (Fig. 1) to detect water transfer between the compartments. Tracers were applied in the early evening (5 P.M.), then the lights in the growth chamber were turned off, as Querejeta et al. (2003) found that water transfer only occurred in their experimental system during the night. Two of the seven mesocosms were "controls" (C1 and C2), in which hyphal connections between the compartments were severed (by running a sharp knife along both sides of the wall that separated the central compartment from the side compartments) immediately before the tracer injection. The controls were included to distinguish movements of the tracers in the soil from transfer via ECM hyphae. Initially, there were three controls, but the hyphae in one of the mesocosms were not completely severed, so it was excluded from the experiment.

Sampling and analyses

The seven mesocosms were destructively sampled the morning after the tracer injections, ca. 15 h after the injection. The growth media in the smaller side compartments (Fig. 1b) were divided into an upper (1; 0–75 mm deep) and lower layer (2; 75–150 mm deep), while the central compartment, where the manzanita was growing, was divided into four 75-mm layers of the same thickness (5 cm) as the side pots. Soil samples were taken from all eight soil layers in each mesocosm to extract hyphae. Subsamples of roots were also taken from all layers for morphology-based classification. Additional root samples for water extractions were taken only from the side compartments and were kept in airtight plastic bottles that were stored in plastic bags and refrigerated (~5°C). After the harvest, xylem sap was extracted from the roots by cryogenic distillation (Ehleringer et al. 2000).

Hyphae were extracted from each soil sample using 3% (*w/v*) sodium hexametaphosphate solution, then filtered through nitrocellulose membranes with 1.2-μm pores (Hanssen et al. 1974) and examined for labelling. The membranes were mounted in glycerol on glass microscope slides and viewed and scored using a Nikon Optiphot-2 microscope equipped with an epifluorescence optical system [flourescein isothiocyanate (FITC) filter set, 460–500 nm excitation, 505 nm dichroic mirror, 510–560 nm emission]. All hyphae were then scored as labelled or non-labelled. Hyphal counts were taken in at least 25 fields of view per slide (200× magnification), and the lengths of labelled and non-labelled hyphae were calculated using the modified line intersect method (Tennant 1975). There was a problem with leakage when filtering the hyphae from two of the mesocosms (one unsevered and one severed), which did not affect the relative proportions of labelled or unlabelled hyphae in each sample, but resulted in the hyphal lengths of these two mesocosms being underestimated; therefore, results from only five mesocosms are shown in Fig. 2.

Root tips were examined and classified on the basis of their gross morphology (10–25× magnification) according to their colour, texture, branching patterns, mantle structure and the presence and abundance of emanating hyphae and/or rhizomorphs (Agerer 1986–1998). The term morphotype is used in this paper to designate a recognizable group of mycorrhizal root tips. Each morphotype was identified by molecular analysis to species level whenever possible. The total abundance (number of mycorrhizal root tips per subsample from each soil layer) and relative abundance (number of each morphotype/total number of living mycorrhizal root tips) of each mycorrhizal type were recorded. Non-mycorrhizal root tips were also recorded. Subsamples of morphotypes (1–10 subsamples of each morphotype) were stored at −80°C before molecular analysis. Internal transcribed spacer–restriction fragment length polymorphism (ITS-RFLP) analysis was used as a supplement to the morphotyping to confirm consistency within and between single morphotypes and to identify the species involved in water transfer. DNA was extracted from both mycorrhizal root tips and rhizomorphs. Fresh tissue was pulverized for 30 s with a 3-mm glass bead in a mini-bead beater (Biospec) and suspended in 1,000 μl cetyltrimethylammonium bromide (CTAB) buffer. After 60-min

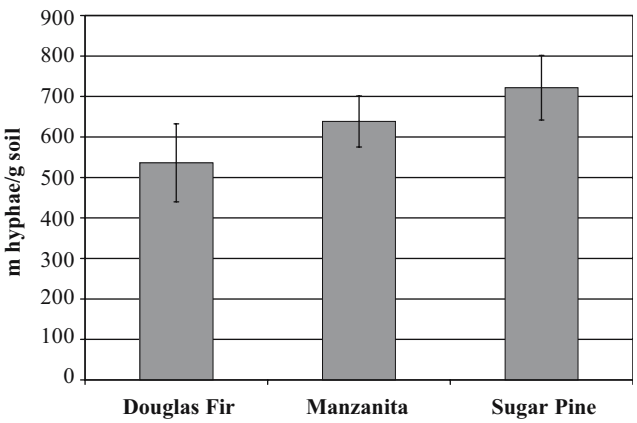

Fig. 2 Hyphal length, in m g^{-1} soil (mean±SE) for each of the different compartments of the mesocosms

lysis incubation at 65°C, total nucleic acids were isolated and cleaned using a Qiagen DNeasy kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. The internally transcribed spacer regions of the recombinant DNA (rDNA) were amplified using the primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). PCR was performed in a PTC-100 thermal cycler (MJ Research, Waltham, MA, USA) in conditions previously described (Gardes and Bruns 1993). Polymerase chain reaction (PCR) products were prepared for sequencing reactions by cleaning with a Qiagen PCR purification kit (Qiagen, Valencia, CA, USA). Single-pass sequencing of the ITS1/5.8S/ITS2 region of the rDNA was performed using an ABI3100 genetic analyser, and the acquired data were analysed with Sequence Analysis 3.4.1 software (Applied Biosystems Foster City, CA, USA). The sequences were then compared to available sequences in the GenBank database (http://www.ncbi.nlm.nih.gov) using basic local alignment search tool (BLAST) search analysis (Altschul et al. 1997). Sequences with similar affinities were then manually aligned to identify similar fungal species in the mesocosms.

The hydrogen isotope ratio of the water was determined using an isotope mass spectrometer (IRMS; Europa Integra.) with a continuous flow design integrated with an on-line sample combustion interface at the UC Davis Stable Isotope Facility. Results are expressed in standard delta notation (δD) in parts per thousand (‰) relative to Vienna Standard Mean Ocean Water (V-SMOW), where $\delta D=[(R_{sample}/R_{V\text{-}SMOW})\text{-}1]\times 1{,}000‰$, and R is the molar ratio of heavy to light isotopes (D/H) with a sample precision of ±1‰.

Statistical analysis

Analysis of variance was performed to check for differences in mycorrhizal morphotypes, hyphal length and extent of root colonization between plant species using the ANOVA procedure of the Statistical Package for the Social Sciences (SPSS) statistical package (version 10, 1999). The correlation between the amount of deuterium in the roots of the seedlings and the amount of labelled hypha and root tips were analysed by regression analysis. Differences were considered significant if their probability level was less than 0.05, unless otherwise stated.

Results

There were substantial amounts of fungal hyphae in each of the compartments of the mesocosms, and there were no significant differences between the different compartments in terms of hyphal length ($p=0.549$; Fig. 2). Rhizomorphs from *Atheliaceae* and *Rhizopogon* fungi were extracted from different compartments and identified by molecular technique. GenBank accession numbers for *Atheliaceae* rhizomorphs found in sugar pine compartments are EF397697 and for Douglas-fir compartments, EF397698, and for manzanita compartments, EF397696. GenBank accession numbers for *Rhizopogon* rhizomorphs found in sugar pine and manzanita compartments are EF397695 and EF397699, respectively. Sugar pine and manzanita seedlings had significantly more mycorrhizal root tips than the Douglas-fir seedlings ($p=0.005$, $F=11.259$; Table 2). After harvesting, we divided the mycorrhizal root tips into 15 different morphotypes, but only six of them were identified. After weighting for differences in percent colonisation, we found that 4 of the 15 morphotypes had colonised >70% of the mycorrhizal root tips in all compartments. The most abundant morphotype in the three different compartments was morphotype 1 (*Atheliaceae*), and 20–35% of all mycorrhizal root tips were infected by this fungus. Morphotype 11 consists of two species, *Phialocephala*

Table 2 Morphotypes linked to fungal species, their GenBank accession number and the mean value and SE of mycorrhizal colonisation (%) for the Douglas-fir, manzanita and sugar pine

Morphotypes	Compartments		
	Douglas-fir	Manzanita	Sugar Pine
1	*Atheliaceae*, EF397680	*Atheliaceae*, EF397682	*Atheliaceae*, EF397681
2	Unknown apacrid root taxa, EF397683		
3	*Phialocephala fortinii*, EF397684		*Rhizopogon evadens*, EF397685
4	*Laccaria*, EF397686		
5	*Phialophora finlandia*, EF397687	*Phialophora finlandia*, EF397688	*Phialophora finlandia*, EF397689
6	*Rhizopogon vinicolor*, EF397690		
7		*Phialocephala fortinii*, EF397691; *Rhizopogon arctostaphyli*, EF397692	*Phialocephala fortinii*, EF397693; *Rhizopogon arctostaphyli*, EF397694
Mycorrhizal colonisation % (SE)	47.5 (7.4)	70.2 (3.8)	85.3 (3.8)

fortinii and *Rhizopogon arctostaphyli*. The morphotype of *P. fortinii* that was found in group 11 is probably a later developmental stage of the darker brown hyphal type that was designated morphotype 3. We were unable to identify eight of the unknown morphotypes, either because they contained DNA from more than one fungal species or because our DNA extraction method was not successful.

Fluorescent dye results

Hyphae labelled with the fluorescent dye (which were readily distinguished from non-labelled and autofluorescent ECM hyphae) were found in both the sugar pine and the Douglas-fir compartments (bars in Figs. 3 and 4b,c). More than half (57%) of the hyphae in the lower soil layer in the sugar pine compartment of mesocosm 2 were labelled, but the proportion of labelled hyphae found in the Douglas-fir compartments was always less than 20% (Fig. 3). We also detected fluorescently labelled mycorrhizal root tips in both sugar pine and Douglas-fir compartments (Fig. 4a), but again, more fluorescent dye was found in the sugar pine than in Douglas-fir root tips (p=0.003, F=17.21; Table 3). Labelled root tips were identified on the basis of abundant LYCH-labelled hyphae on the root surface (Fig. 4a), while non-labelled root tips only displayed a pale blue autofluorescence signal (not shown). Notably, only one fungal morphotype (*Atheliaceae* morphotype 1) was found on the root tips with rhizomorphs that were labelled with the fluorescent dye.

Isotope labelling results

In contrast to our fluorescent dye results, the enriched deuterium-labelled water (HDO) was only found in Douglas-fir roots in four of the five mesocosms where the hyphal connections were not severed (diamonds; Fig. 3). If we use our deuterium data to estimate the amount of water transferred overnight, we find that a very small proportion (0.01–0.04%) of the total volume of water extracted from the Douglas-fir root tissues is transferred from the central compartment. The proportion of deuterium label in the seedlings corresponded to neither the amount of fluorescently labelled hyphae (r^2=0.0014) in the mesocosm nor the amount of fluorescently labelled root tips (r^2=0.1653; Fig. 3 and Table 3).

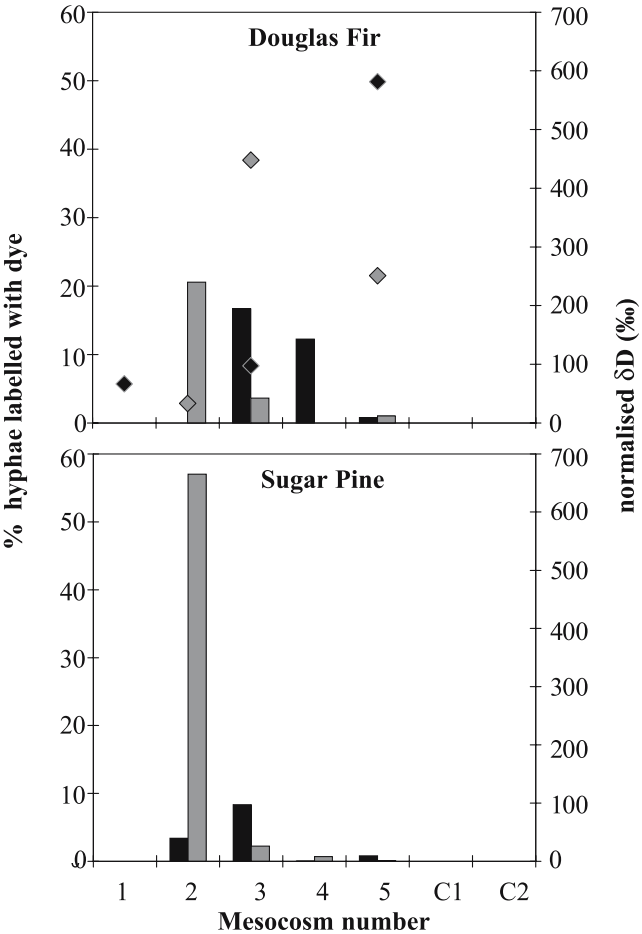

Fig. 3 Percentages of the fungal hyphae labelled with fluorescent dye in the shallow (0–75 mm depth, *black bars*) and deep (75–150 mm depth, *grey bars*) soil layers in the Douglas-fir and sugar pine compartments, and normalised deuterium abundance (in relation to the control mesocosms, ‰) in Douglas-fir roots from the shallow (*black diamonds*) and deep (*grey diamonds*) soil layers

Discussion

The analyses using the two different tracers in this study yielded contrasting results. Fungal hyphae were clearly associated with the roots of the conifer seedlings in both compartments containing such seedlings, and these hyphae were labelled with fluorescent dye. However, the dye had only moved through one fungal group, the *Atheliaceae*, which were associated with both of the conifers. These findings show that the capacity to transport the dye differs between mycorrhizal fungal species and illustrate the importance of including an appropriate mycorrhizal community in laboratory-based experiments when studying transfer via fungal hyphae. The fact that the dye was transferred to both conifer species but the isotope-labelled water was not raises several methodological considerations. Firstly, our results raise the possibility that the dye and isotope tracers may be transferred by different mechanisms, which may be due to differences in the identity of fungal species that were found colonising the roots of the two trees species (Table 2). The dye was seen to have moved through only one group of fungi, the *Atheliaceae*, rhizomorphous fungal species that were found to be associated with both of the conifers. Three different *Rhizopogon* species, *Rhizopogon vinicolor*, *R. evadens* and *R. arctostaphyli*, which also

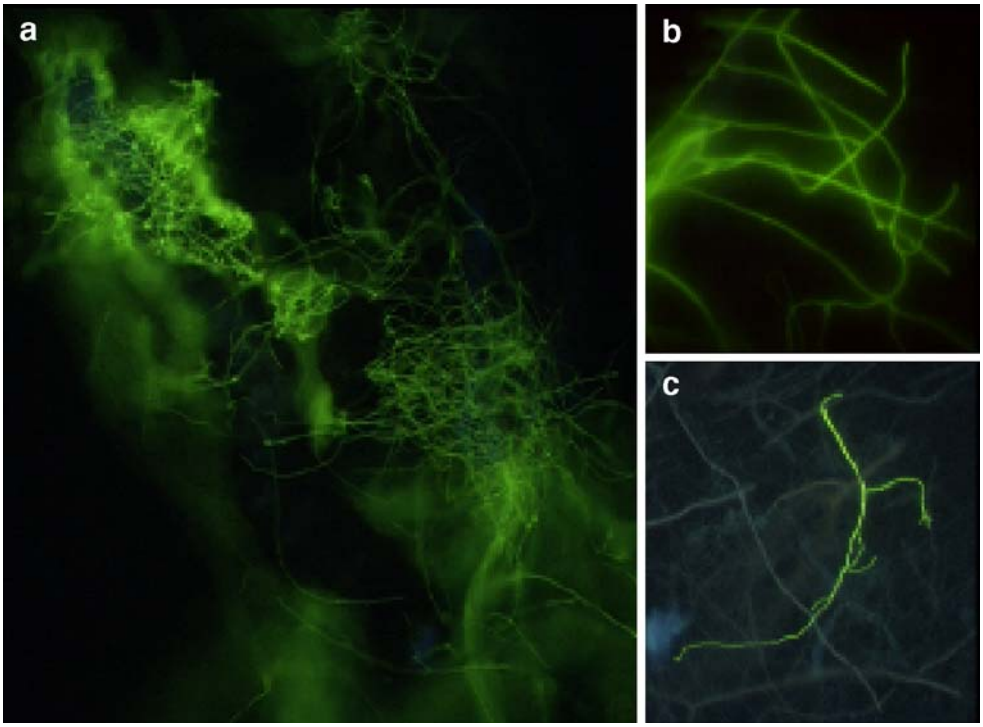

Fig. 4 a–c Mass of external ECM hyphae labelled with lucifer yellow carbohydrazide (LYCH, bright yellow-green fluorescence) either surrounding several root tips exhibiting weak autofluorescence (*pale blue*) (**a**) or free-labelled hyphae within the soil profile (**b**). LYCH-labelled (*green*) and unlabelled, autofluorescent (*pale blue*) hyphae within the same sample (**c**)

have rhizomorphs, were abundant in the mesocosms; however, no dye was seen to have moved through these rhizomorphs. Agerer (2001) suggested that fungal species possessing long and sometimes differentiated hyphae and rhizomorphs are suitable for transferring water and solutes over relatively long distances. Increased deuterium concentrations were only found in Douglas-fir roots, not in sugar pine roots, indicating that water transfer could have occurred via fungal species other than those involved in the dye transfer that did not colonise our sugar pine seedlings. However, weighting for the proportions of fungal hyphae and mycorrhizal root tips does not help explain these results (data not shown). Secondly, the reason why the deuterium-labelled water and the fluorescent dye may have been transferred differently could be related to differences in the loading and transfer pathways involved, as LYCH is strictly an apoplastic tracer, moving only via cell wall pathways, whereas water will move via both the apoplast and intercellular pathways. The presence of dye in both receiver species indicates both the presence of apoplastic bypasses for the transfer of water-soluble low molecular weight compounds (LYCH, MW 457) at the fungal–root interface and low hydraulic resistance to the apoplastic transfer of solutes via fungal hyphae and rhizomorphs. These findings were not unexpected, as the apoplastic pathway controls the transfer of solutes between symbiotic partners (Ashford et al. 1989), and the apoplastic movement of dye has been previously detected in mycorrhizae (Querejeta et al. 2003). However, the finding that HDO (deuterium-labelled water) was transferred via fungal hyphae to Douglas-fir, but not sugar pine, seedlings implies the presence of an additional water transfer mechanism.

As HDO transfer occurred during the night, when transpiration had ceased, it is likely that solutes and nutrients in the roots created an osmotic gradient which, in turn, established a water potential gradient that was large enough to move water from fungi to plants via an intercellular water transport pathway (Steudle 2000a,b) in Douglas-fir, but not sugar pine. Differences in water transfer patterns have been explained previously by differences in root anatomy (e.g. Peterson et al. 1993; Steudle et al. 1993). Not only convolutions within the fungal tissue (Hartig net) but also the elongation of the cortical cells helps increase the surface area for transfer (Barker et al. 1998). In addition,

Table 3 Number and percentage of Douglas fir and sugar pine root tips labelled with fluorescent dye in the seven mesocosms

Root tips		1	2	3	4	5	C1	C2
Douglas fir	Number	12	1	3	7	3	0	0
	Percent	4	2	3	7	6	0	0
Sugar pine	Number	7	2	105	65	21	0	0
	Percent	4	5	92	36	27	0	0

the development of ectomycorrhizae might up-regulate the activity of aquaporins, a sort-of protein-based "gateway" that regulates intercellular water transfer, as observed in arbuscular mycorrhizae (Krajinski et al. 2000). Nevertheless, in our study, Douglas-fir and sugar pine seedlings were colonised to similar extents by ECM fungi, so differences in root morphology alone do not explain the differences in HDO transfer between the two receiver plant species.

We believe that despite some differences, both methods provide important information about resource transfer in plant rhizospheres. We cannot be sure that the dye transfer data provide accurate indications of water transfer rates and patterns; however, our results show that both dye and labelled water do move between fungi and plant roots.

Previous investigations have detected the transfer of various molecules including N compounds (Finlay et al. 1989; Ek et al. 1994), P (Melin and Nilsson 1950; Finlay and Read 1986b; Rousseau et al. 1994) and nutrients such as Ca, S and Zn (Harley and Smith 1983) via mycorrhizal fungi. It has also been suggested in several laboratory-based experiments that carbon is transported between plants connected via ECM and vesicular-arbuscular mycorrhiza (VAM) networks (Read et al. 1985; Finlay and Read 1986a; Simard et al. 1997a; Watkins et al. 1996; Fitter et al. 1998). Simard et al. (1997b) and Lerat et al. (2002) also showed transfer of C in the field. The overall biological significance of nutrient uptake and transfer to plants via fungal hyphae is still not fully understood because the transport rates and quantities are difficult to estimate and may differ between field and laboratory-based experiments.

In natural plant communities, *Arctostaphylos* shrubs and conifer seedlings co-occur. Some mycorrhizal fungal species form more than one category of mycorrhizae depending on the plant associate involved. For example, some basidiomycetous and ascomycetous fungi that form ectomycorrhizae with trees form arbutoid mycorrhizae in association with ericaceous plants of the genera *Arbutus* and *Arctostaphylos* (Zak 1976; Molina and Trappe 1982; Acsai and Largent 1983). Although the seedlings may have benefited directly from fungal linkage to *Arctostaphylos*, mycelial networks are beneficial to newly establishing seedlings even in the absence of direct linkage and water transfer between plant species. Seedlings that associate with an established fungal mycelium may not need to allocate any resources to produce fungal biomass (Newman 1988). However, there are still many unanswered questions that need to be addressed if we are to understand the importance of mycorrhiza in water transport; for example, how does the transport capacity for various compounds differ between different fungal species and how much water is transported?

Our results show that both tracers used in this study can provide information on transfer between fungi and plants, although dye transfer data may not provide accurate indications of water transfer rates and patterns. The isotope method provides a more direct measure of water movement and is therefore preferable when studying water relations of plants and their associated mycorrhizal fungi. The stable isotope method also allows quantitative estimates of water uptake and transfer to be made using standard mixing model equations (Dawson et al. 2002).

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