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Yu-Ping Sun · Torgny Unestam · Steven D. Lucas
Karl Johan Johanson · Lennart Kenne · Roger Finlay

Exudation-reabsorption in a mycorrhizal fungus, the dynamic interface for interaction with soil and soil microorganisms

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Abstract The mycelium of *Suillus bovinus* slowly absorbed [U-^{14}C]glucose and other tracers from droplets placed on the cords, translocated them to the peripheral hyphae and exuded them into fluid drops on the hyphal tips. The exudate was characterized by ^{1}H NMR spectroscopy and by sugar and amino acid analysis. The exuded compounds were mainly carbohydrates and peptides. Acetic acid and oxalic acid were also present in the exudate along with a number of unidentified compounds. Released ions (K, Na, Cl, P, Mg and Ca) were identified by X-ray microanalysis. The mycelium was shown to reabsorb up to 65% of the exuded ^{14}C compounds in 2 days. Glucose, mannitol, glutamic acid (pH 3.2), and Rb^+ (as well as other mineral ions) were all readily absorbed by the mycelium, while oxalic acid at pH 4.2 and glutamic acid at pH 6.5 were not. Exudation of fluid droplets on the surface of the hydrophobic mycorrhizal fungus *S. bovinus* may represent an ecophysiologically important function of the extramatrical hyphae, which provides an interface for interaction with the immediate hyphal environment and its other microorganisms where the peripheral hyphae exchange their photosynthetically derived products for nutrients to be used later by the pine host. We hypothesize that actively absorbed carbohydrates from the root are translocated to the peripheral hyphae along a concentration gradient of sugars and polyols by means of active translocation and diffusion in cell elements and by acropetal water transport in the cord vessels.

Key word Exudation · Reabsorption · Mycelium-soil interface · Osmotic gradient pump · Carbon and water translocation

Y.-P. Sun · T. Unestam (✉) · R. Finlay
Department of Forest Mycology and Pathology,
Swedish University of Agricultural Sciences,
P.O. Box 7026,
S-750 07 Uppsala, Sweden
e-mail: torgny.unestam@mykopat.slu.se

S.D. Lucas
Department of Surgery, Uppsala University,
S-751 85 Uppsala, Sweden

K.J. Johanson
Department of Radioecology,
Swedish University of Agricultural Sciences,
P.O. Box 7031,
S-750 07 Uppsala, Sweden

L. Kenne
Department of Chemistry,
Swedish University of Agricultural Sciences,
P.O. Box 7015,
S-750 07 Uppsala, Sweden

Introduction

Organisms need to be able to discard waste products. This is true of the simplest unicellular life forms up to the most complex mammals, all of which need to excrete water and metabolites selectively while maintaining internal homeostasis. Bacteria and protozoa have a number of mechanisms for exuding waste products through the cell membrane, thereby depositing them in and influencing the external environment (Nisbet 1984; Brock and Madigan 1991). Most plants cells use additional strategies, such as storing non-degradable waste in intracellular vacuoles (Ridge 1991). Fungi use their vacuoles for many tasks (Klionsky et al. 1990) but irreversible storage of waste products has not been shown. Instead they may rely to a considerable extent on the exudation of by-products, such as oxalic acid (Unestam and Sun 1995), across the plasma membranes and cell walls, which thus act as molecular sieves (Jennings and Bravery 1991; Wessels l993 and references therein).

So-called "tear drops" have previously been seen on hyphae of *Serpula lacrimans* (Jennings and Bravery 1991). Unestam and Sun (1995) found that such droplets were exuded from the extramatrical hyphae of many hydrophobic mycorrhizal fungi. When hyphae grew into oil, tiny droplets also appeared on hydrophilic fungi as the oil prevented their removal by the

capillarity of the hyphal walls. Unestam and Sun (1995) suggested that such droplets represent an excretory process in the fungal hypha.

This present study explores this hypothesis and presents evidence for exudation-reabsorption in the peripheral, extramatrical mycelium of an ectomycorrhizal fungus, *Suillus bovinus* (L. ex Fr.) O. Kuntze. The main objective was to evaluate the physiological basis of this hypothesized function in the peripheral extramatrical mycelium of the mycobiont. The exudation-absorption process may represent an important exchange through the interface to adjacent soil and microorganisms, thereby conditioning the immediate hyphal environment. In a parallel study (Unestam et al. 1999), we have discussed the possible function of the hydrophobic mycorrhizal mycelium in soil. In this paper, we present and discuss the possible significance of results obtained in vitro.

Materials and methods

Fungal strain

The mycorrhizal fungus *S. bovinus* (Strain L1) used in this investigation was the same as that previously studied by Unestam and Sun (1995).

Fluid drop exudation

S. bovinus grew on cellophane-covered MMN nutrient agar medium at 20 °C in darkness for 21 days. In repeated experiments, several "trap" drops (in total 4 µl or more) of sterilized, distilled water were pipetted onto the hydrophobic aerial hyphae behind the mycelial margin to capture products exuded through the cell wall. After 1–2 weeks, the added water was collected by means of a micropipette, pooled in a small plastic vial and stored at –20 °C. In a typical experiment, the dry weight of the sample (17.5 ml) was 12.5 mg.

NMR spectroscopy

The sampled and bulked water drops of *S. bovinus* (pH about 6.5) were collected, freeze-dried and dissolved in 1 ml deuterium oxide (D_2O). A 0.7 ml aliquot was transferred to an NMR-tube after filtration through glass wool. The ^{1}H-NMR spectrum was obtained at 30 °C on a Varian VXR-400 spectrometer. The chemical shifts were given relative to the HOD signal (d 4.70). The pD value of the sample in D_2O was determined by a glass pH microelectrode (Metrohm pH-Meter). Standards used were mannitol, trehalose and arabitol (Brownlee and Jennings 1981a), amino acids (glutamic acid/ glutamine, aspartic acid /asparagine and alanine, with the same pD as the sample) (Finlay et al. 1988), and sodium acetate, all of analytical grade.

Sugar analysis

The relative proportions of free sugars and polyols were estimated by gas-liquid chromatography-mass spectrometry (GC-MS) as the corresponding alditol acetates (Sloneker 1972) using a Hewlett-Packard 5890 gas chromatograph and 5970 mass selective detector. A solution of part of the exuded sample (1.5 mg) was treated with $NaBD_4$ (3 mg in 0.3 ml 1 M ammonia) at 40 °C for 30 min, made acidic with acetic acid and evaporated to dryness. To remove the boric acid, the product was dissolved in methanol (0.5 ml) containing 10% acetic acid and the solution evaporated to dryness. This procedure was repeated three times. Thereafter, the sample was acetylated with acetic anhydride in pyridine (1:1, 0.3 ml) at 120 °C for 20 min and the solution evaporated to dryness. Toluene (0.5 ml) was then added and the solution evaporated to dryness. The products were dissolved in methanol (0.1 ml) and a 6 µl-aliquot was injected into the GC. To differentiate between fructose and a mixture of glucose and mannose, another aliquot of the exuded sample was analysed as described above but without the $NaBD_4$ step.

Amino acid analysis

In each experiment, the sample was hydrolysed in 1 ml 6 M HCl containing 500 nmol norleucine as an internal standard. After evaporation of the HCl, the hydrolysate was dissolved in 1.0 ml of application buffer and 20 µl was applied to the column of an (a+)-amino acid analyser (LKB Instrument).

Analysis of inorganic compounds

The ions in the samples used for NMR spectroscopy were analysed according to the methods described by Quinton (1978) and Jacobsson (1994). The samples (80 pl) dried on copper grids (150 mesh) were analysed at 100 kV in the transmission mode using a Philips 400 transmission electron microscope with a field emission gun, and a LINK QX200 energy-dispersive X-ray micro-analysis system. References samples of K, Na, Cl, PO_4, Mg, and Ca (in six dilutions) were used.

Labelled compounds

The following compounds were employed in the study: D-[U-^{14}C]glucose at a specific activity of 0.12 GBq/mmol (DuPont NEN); D-[1-^{14}C]mannitol at a specific activity of 2.07 GBq/mmol (Amersham); L-[U-^{14}C]glutamic acid at a specific activity of 9.7 GBq/mmol (DuPont NEN); [U-^{14}C]oxalic acid at a specific activity of 0.19 GBq/mmol (DuPont NEN); ^{86}Rb at a specific activity of 0.11 GBq/mg (DuPont NEN).

Labelling experiments

Empty Petri dishes were inoculated with agar plugs (5×5 mm) of *S. bovinus* mycelium before adding 0.1 ml of liquid MMN medium to the inoculum. Several blocks of water agar without mycelium were placed at the edges inside the dish to maintain humidity. The dishes were sealed with a double layer of Parafilm and kept in a plastic container with water-saturated air at 20 °C in darkness for 6–8 weeks.

Before adding the labelled compound, the mycelium growing on the plastic surface was separated using a scalpel into 8 radial sectors still in contact with the inoculum in the centre. All experiments were conducted with unsealed Petri dishes placed in the centre of a desiccator. In order to minimize fixation of any $^{14}CO_2$ released by respiration, a gentle current of water-saturated air was passed over the mycelium from the edge of the dish and aspirated through a tube at the centre of the lid. The labelling experiments were run for 2 days and the dishes were then dried at 70 °C for 20 h before autoradiography.

In the exudation experiments, 4 µl of a 35 mM glucose solution (containing l8.5 kBq [U-^{14}C]glucose) was pipetted on to a sector of mycelium as a drop that stayed intact for days at a distance of 5–7 mm from the inoculum plug, i.e. less than halfway to the mycelial margin. Trap droplets (each about 4 µl of distilled water) were added to the surface of the marginal mycelium about

20 mm distally from the labelling site within the same sector. The droplets were collected and replaced by new drops every hour, bulking the removed droplets.

In a reabsorption-redistribution experiment, 4 μl (about 5500 cpm) of the bulked sample was then added as a labelling source onto the mid-region of a sector, i.e. about 10 mm from the margin of an unlabelled mycelium.

For absorption experiments with other organic compounds and ^{86}Rb (see below), activities were 5000–6000 cpm per 4 μl added to the mid-region. In some cases, a droplet of sodium azide (0.01 M), commonly used as a respiration inhibitor, was added to the mycelium about 3 mm further out from the labelled droplet.

Loss of nuclide into the desiccator atmosphere in the form of aerosol was estimated using a nuclide blanks run on the plastic surface without mycelium and the results adjusted accordingly.

Each experiment consisted of five replicates and the results were expressed as the percentage (±standard error) of the absorbed labelling compound (i.e. activity lost per labelling droplet minus blank).

Determination of the activity of nuclides

^{14}C and ^{86}Rb activities were determined using a liquid scintillation counter (Packard Instrument Company). A drop (0.03 ml) of exudate was mixed with 6 ml of scintillation solution (Quicksafe A, Zinsser Analytic) in a plastic vial. The measuring time was chosen to obtain results with less than 2% error (S.D.) due to random processes of decay.

Macroautoradiography

Macroautoradiography was used to visualize the distribution of ^{14}C and ^{86}Rb in mycelia in vitro. The dried mycelium on the Petri dish was covered with X-ray film (SB type, Eastman Kodak, Rochester, N.Y.). Exposure was at room temperature for 2 days and the film was developed in Kodak D19B and fixed in Kodafix.

Phosphor image plates (Fuji) were also used. The plates were erased with white light (500 nm, Osram, Cool White) for 20 min and exposed for 2 h. The plates were then analysed on a Phosphor Imager (Molecular Dynamics) and the image adjusted using the Image Quant software supplied with the apparatus.

Formation of calcium oxalate crystals

Petri dish cultures, as in the labelling experiments, were used. A drop of 2% glucose was added at regular intervals to the surface of the mycelium colonizing the central agar plug. Drops of of $CaCl_2 \cdot H_2O$ (5 g/l) were also added to the mycelial margin for microscopic studies of calcium oxalate crystal formation on the hyphae. Commercial crystals and those analysed by transmission electron microscopy-Xray (Unestam and Sun 1995) were compared.

Results

Analysis of exuded compounds

K, Na, Cl, P, Mg and Ca ions, trapped in added distilled water drops, were found by X-ray microanalysis, and their concentrations in a representative sample were 5.9, 1.5, 1.2, 0.6, 0.2 and 0.002 mM, respectively.

Figure 1 shows the ^{1}H NMR spectrum of the diluted exudate after transfer into D_2O (pD 3.6). According to the chemical shifts in the ^{1}H NMR spectrum, carbohydrates and peptides were dominant in the droplets. By comparison with standard references, only acetate (d 1.95 ppm) could be identified.

Table 1 shows the major sugars and polyols in the diluted exudate. Inositol, erythritol, mannitol, threitol, xylitol and ribose were the dominant soluble carbohydrates. By using $NaBD_4$ as the reducing agent, it was possible to differentiate between the alditols and the sugars, as the latter showed an extra mass unit in the mass spectra derived from a deuterium in the 1-position. The alditol acetate from the unknown hexose had the same retention time as that of allose, but the structure of allose was not confirmed.

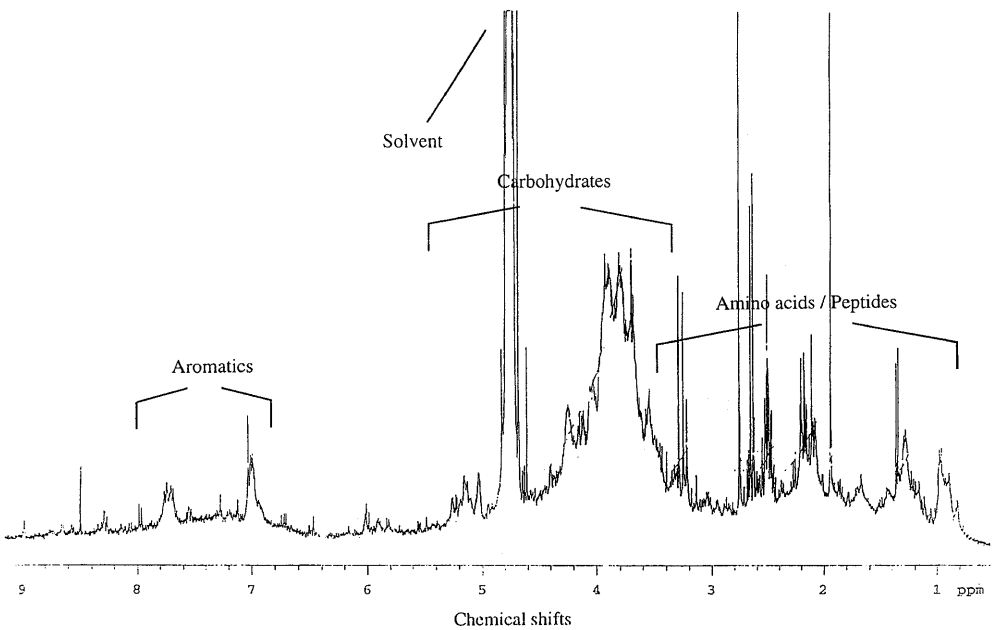

Fig. 1 A ^{1}H NMR spectrum of exudate from *Suillus bovinus*. Water drops added onto the hydrophobic mycelium as traps for fungal exudate were collected and pooled

Table 1 Sugars and polyols exuded by *Suillus bovinus*, which made up 32% of the exudate mass

Compounds	% Exudate mass
Inositol	9.7
Erythritol	4.9
Ribose	4.0
Threitol	3.5
Xylitol	3.0
Mannitol	2.8
Arabinose	1.6
Arabitol	1.0
Glucose	0.9
Hexose	0.7

Table 2 Amino acid composition of peptides exuded by *S. bovinus* which made up 14% of the exudate mass

Component	% Exudate mass
Glycine	3.8
Glutamic acid	2.5
Aspartic acid	2.4
Proline	1.7
Alanine	1.1
Serine	0.8
Threonine	0.4
Leucine	0.4
Arginine	0.4
Valine	0.3
Phenylalanine	0.3
Isoleucine	0.2
Tyrosine	0.1

Peptides were present but no free amino acids were detected. After hydrolysis, thirteen different amino acids were identified (Table 2).

The trap droplets on the mycelium gradually turned brown within a few days, indicating release of phenolic compounds.

Exudation of organic compounds

The hyphae of the proximal part of the mycelium were able to absorb [U-^{14}C]glucose and translocate ^{14}C compounds in the cords, finer strands, and hyphae to the growing tips (about 10 mm/24 h). ^{14}C compounds were exuded through hyphal cell walls into the added water drops and the ^{14}C activity increased steadily with time. The varied concentration of the ^{14}C activity reflects the varying ability of the mycelium to take up glucose, translocate the labelled metabolites, and exude the different labelled compounds.

When the respiratory inhibitor sodium azide (Jennings 1995) was added as a drop onto the mycelium equidistant (3 mm) from the [U-^{14}C]glucose source and the sink water drop, an immediate decrease in the exuded ^{14}C activity was observed in the trap drops. After 1 h, exudation by the mycelium had decreased by 50% and after 2 h by 85%. After 3–4 h, the hyphal tips had released almost no ^{14}C compounds. Addition of azide immediately behind the peripheral hyphal tips did not noticeably affect exudation.

Absorption of ^{14}C-labelled compounds

The mycelium absorbed 45 ± 8% of the added [U-^{14}C]glucose during the first 50 h. The experiment using [1-^{14}C]mannitol and [U-^{14}C]glutamic acid (at pH 3.2) demonstrated less absorption (32 ± 7% and 25 ± 5%, respectively) than for [U-^{14}C]glucose. No absorption of [U-^{14}C]glutamic acid was found at pH 6.5.

When [U-^{14}C]oxalic acid (pH 4.2) was used, no ^{14}C compounds were exuded into the trap water drops on the hyphae. An autoradiogram (not shown) confirmed that the mycelium did not absorb and translocate [U-^{14}C]oxalic acid. No capillarity action occurred along hyphal cell walls and no diffusion took place on the plastic surface.

Absorption of ^{86}Rb

Three hours after the addition of ^{86}Rb (pH 3.5, in sodium phosphate buffer), 13 ± 5% of the compound was taken up. The mycelium absorbed 57 ± 6% within 24 h and 73 ± 5% within 50 h. X-ray film autoradiography and phosphor imaging showed high ^{86}Rb activity in the cords and exuded fluid drops 10 mm away within 12 h (as with ^{14}C) (Fig. 2). Much like ^{14}C release, ^{86}Rb exudation was strongly inhibited by azide added close to the source.

Reabsorption of exuded ^{14}C compounds

When ^{14}C-compounds originating from labelled glucose metabolized in the mycelium and exuded by hyphal tips (Fig. 1) were added to the middle of sectors of the new mycelium, 65 ± 6% of the nuclide was reabsorbed during the first 50 h. At higher activities, the mycelium reabsorbed more ^{14}C compounds within the same time span, illustrating that uptake was not the limiting factor in translocation. The varying ^{14}C activity reflects variation in the ability of the mycelium to take up glucose, translocate the labelled metabolites, and exude the different labelled compounds.

The compounds were translocated inside the mycelium from the new labelling source and were carried in both directions. They appeared in the droplets of growing hyphal tips, as shown in Fig. 2. Autoradiograms demonstrated that no diffusion occurred between the cut hyphae of neighbouring sectors (not shown in Fig. 2). Table 3 summarizes the absorption and exudation of compounds in *S. bovinus*.

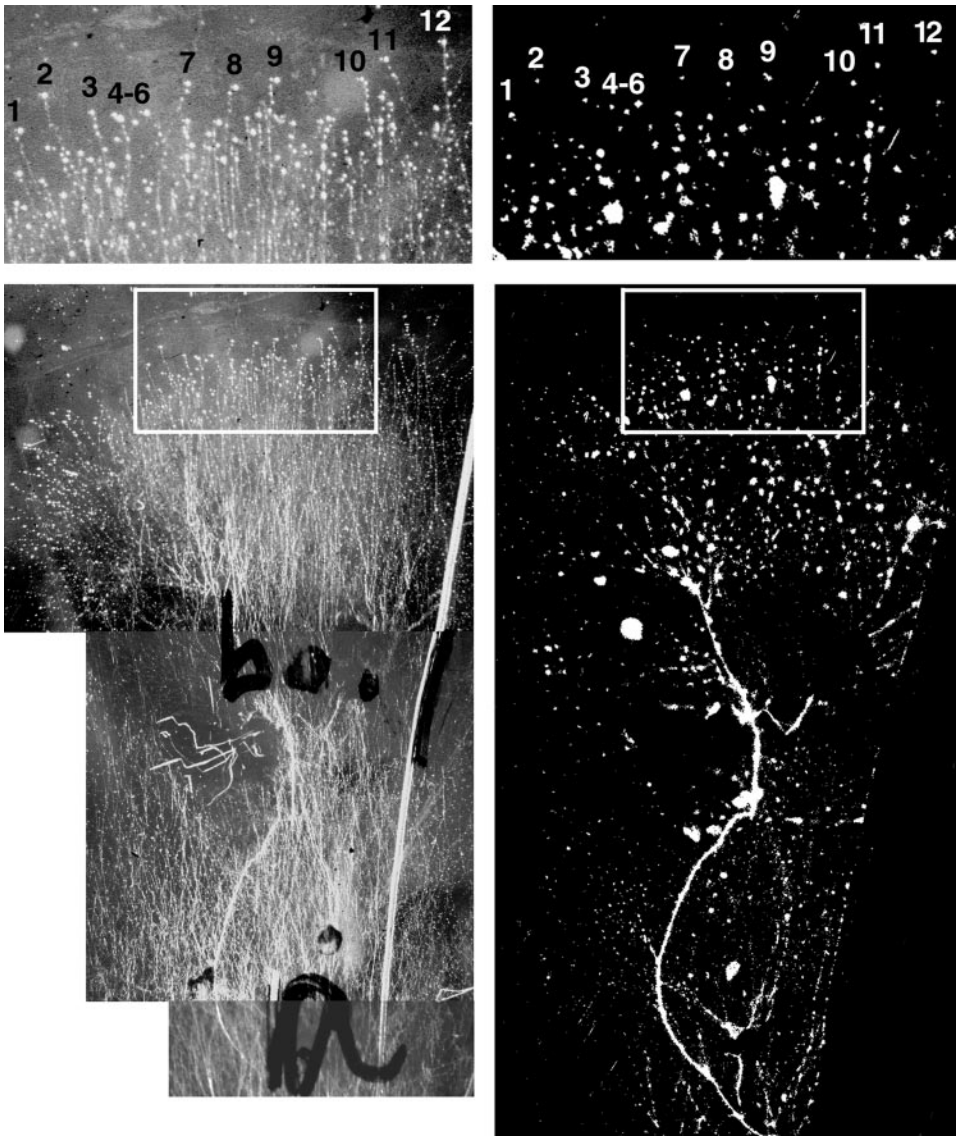

Fig. 2 Photomicrograph of a mycelium (*left*) of *S. bovinus* on a plastic surface and an autoradiogram (*right*) of the same mycelium after addition of labelled droplets (sampled from another labelled mycelium). Reabsorption, translocation and exudation of ^{14}C compounds is shown. No diffusion occurred between the cut hyphae of neighbouring sectors. The heavily labelled, exuded droplets, seen as dots (1–12, *right*), were identical to the exuded water droplets on the hyphal tips (1–12, *left*)

Crystal formation on the hyphal surface

Shortly after the addition of glucose to the inoculum and small drops of $CaCl_2$ at the mycelial margin, calcium oxalate crystals were readily observed under the microscope (Fig. 3). Typical crystals were rarely present on the hyphal tips on the plastic without addition of Ca^{2+} ions.

Discussion

Translocation of water: pump, sources and sinks

Jennings and co-workers (Jennings 1995) studied translocation processes in hyphae of the saprophyte *Serpula lacrimans* and compared it with that in other cord-forming fungi. They concluded that a pressure-driven mass flow of solution dominated in the cords and may even be important for integrated function in an extensive mycelium, where osmotic and other source-sink gradients forward signals in the symplastic-apoplastic system. Centripetal flow in the symplastic hyphae from the exploiting hyphal tips to a carbon source (e.g. a root, wood block) distributes captured minerals to

Table 3 Absorption and exudation of various compounds by *S. bovinus* (+ recorded, − not found, *Nt* not tested)

Exuded		Absorbed	
Sugars and polyols	+	[1-^{14}C]mannitol	+
		[U-^{14}C]glucose	+
Peptides	+	[U-^{14}C]glutamic acid (pH 3.2)	+
		[U-^{14}C]glutamic acid (pH 6.5)	−
Oxalic acid	+	[U-^{14}C]oxalic acid (pH 4.2)	−
Acetic acid	+		Nt
H_2O	+	H_2O	+
K^+	+	K^+ (as ^{86}Rb)	+
Na^+, Cl^-, (PO_4^{3-}),	+		
Mg^{2+}, Ca^{2+}	+		

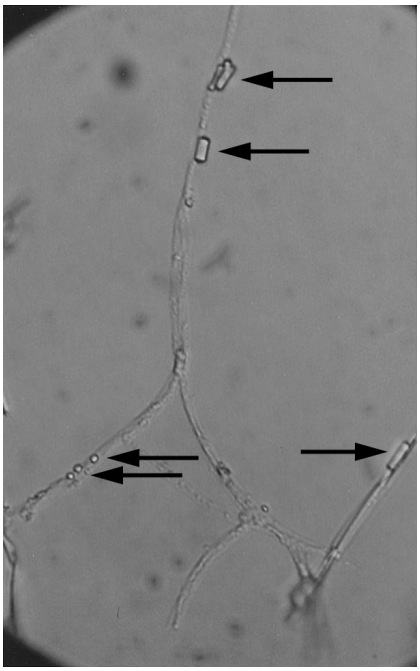

Fig. 3 Typical calcium oxalate crystals (*arrows*) formed after addition of $CaCl_2$ to the hypha

sinks in the mycelium associated with the carbon source. In other hyphae, an acropetal flow from the carbon source is driven by cytoplasmic streaming and mass flow brings solutes out to the peripheral cord hyphal tips, where water droplets often form on the hyphal surface.

In our in vitro system, the loading of a glucose solution may lead to continuous water absorption from available sources, such as water-saturated mycelial parts, so that mass flow towards the hyphal tips occurs and droplets form. The possibility cannot be excluded that some water uptake as well as sugars by the fungus in the mycorrhizal association takes place in the mycorrhizal root. Roots of plants are known to exude water under conditions of low light (Williams et al. 1993), so excess water could very well be available to the mycelium from the mycorrhizal roots. The hydrostatic pressure thus created may drive water and solutes through functioning hyphae, cord vessels and vacuolated hyphae (Duddridge et al. 1980; Eamus et al. 1985; Cairney 1992; Wells et al. 1995) and, with the help of subsequent, water-attracting polyol formation in the tips (see below), towards the peripheral mycelium. There, particularly in dry soil, water may be lost by exudation, evaporation (Unestam and Sun 1995) and capillary action (Unestam et al. 1999). The exuded liquid would affect growth and survival of bacteria associated with the mycorrhizal hyphosphere, in addition to supplying nutrients.

Despite their presence in extracted mycorrhizal mycelia (Söderström et al. 1988; Finlay et al. 1989), neither free amino acids nor trehalose were detected in droplets exuded by *S. bovinus* in vitro. Cellular pools of these compounds were presumably low or absent, or very active reabsorption specifically removed these but not other exuded solutes. Such an active porter seems especially unlikely for glutamic acid (Chalot et al. 1995) at a pH as high as 6.5. The osmotically very potent mannitol and other polyols were, however, present and may accumulate in the metabolically active hyphal tips. The absence of trehalose at the hyphal tip may indicate a metabolic shift towards polyols for the sake of turgor build-up and exudation.

The decrease in ^{14}C exudation from *S. bovinus* mycelium within 1 h after addition of the respiration inhibitor sodium azide close to the point of application confirms that energy is required for active uptake of sugar and, thereby, for osmotic pressure and turgor build-up. Cellular resorption of selected chemicals at the tip also requires energy (Alberts et al. 1989). However, when ^{86}Rb was added at the food base, together with 0.5% glucose, and azide immediately behind the mycelial front, labelled droplets were still formed at the front within 40 h (unpublished results). Turgor was apparently maintained even though absorption was inhibited.

Sodium azide and oligomycin at the food source also inhibited droplet production by hyphal tips in *Serpula lacrimans* growing on a non-absorbing surface (Coggins et al. 1980; Brownlee and Jennings 1981b). In addition, high concentrations of glucose or KCl added to the same site inhibited translocation, strongly suggesting that osmotic uptake of water at the food source was inhibited. Therefore, active loading and build-up of osmotic pressure seem necessary for acropetal mycelial water translocation (Wells et al. 1995).

In the peripheral hyphae, water translocation, whatever the mechanism, reaches dead ends and is eliminated through hyphal tips. The surplus water and its solutes would be discriminately exuded through the cell membrane and cell wall according to size and charge and end up in fluid droplets or other extracellular water just behind the hyphal tips (Jennings 1991; Unestam and Sun 1995). At the same time, sugars, amino acids and other low-molecular weight metabolites would be selectively and actively reabsorbed by the hyphae, exploited by other microorganisms or absorbed by dry soil (Unestam et al. 1999).

S. bovinus is a hydrophobic mycorrhizal fungus. The exuded water drops, therefore, remained intact after exudation from the hyphae growing on a plastic surface, in the air, or into oil drops, as long as the fungal cell was turgid (Unestam 1991; Unestam and Sun 1995). In the field they are apparently removed by capillarity to adjacent soil particles and become available to bacteria (Unestam et al. 1999). This fluid exudation may be an important prerequisite for the functions of *S. bovinus* in soil and may represent regulated interface exchange between peripheral hyphal tips and their immediate environment. The water-repelling capacity of the mycelium even makes surrounding soil less permeable to water (Unestam et al. 1999) and the exuded fluid will

further condition this soil and influence the composition of the bacterial community associated with the hyphal tips.

In the fungal cell, most of the released ions, sugars, amino acids and other organic catabolites are probably soon reabsorbed by the hyphae (Alberts et al. 1989). The "unwanted" secondary metabolites, such as oxalic acid and phenolics, seem to remain unabsorbed (Unestam and Sun 1995). Cell membranes normally sieve water and solvents indiscriminately, but allow passage according to molecular size, hydrophobicity, and charge. Compounds smaller than 20 kDa may pass fungal cell membranes and cell walls (Wessels 1993). The closest analogy would be the animal kidney where the sieving level is 60 kDa (Schmidt-Nielsen 1983; Junqueira et al. 1986). All organisms in soil need such selective excretion to cope with their own waste products, but in fungi this essential process has been, to our knowledge, completely overlooked.

Trehalose, the major soluble carbohydrate translocated in the mycelium of *Serpula lacrimans* (Brownlee and Jennings 1981a) and probably in the mycelium of *Agaricus bisporus* (Hammond and Nichols 1976), was absent from the hyphal droplets of *S. bovinus*, while several other sugars and polyols were exuded in the droplets. The exuded soluble carbohydrates seem to be readily taken up and utilized by fungal hyphae (Hacskaylo 1973). Amino acids are known to serve as a nitrogen source for ectomycorrhizal fungi (Abuzinadah and Read 1988; Finlay et al. 1989). This concurs with the fact that [U-^{14}C]glutamic acid was absorbed by hyphae of *S. bovinus*.

Despite the importance of K in the adjustment of solute potential in many organisms (Alberts et al. 1989), it seems clear that polyols such as mannitol and arabitol are predominant factors in maintaining turgor pressure in fungi (Griffin 1981; Carlile and Watkinson 1994). Amino acids are also important in regulating the solute potential in bacteria (Griffin 1981). All these key compounds are reabsorbed from the hyphal droplets by *S. bovinus*, implying in essence a selective excretory process. In mycorrhizal fungi, the function may primarily take place in the actively metabolizing hyphae, where the turgor is maintained and the hyphae grow both in dry and moist environments (Unestam and Sun 1995). Whether turgor is also fully responsible for hyphal extension at the thin-walled and dynamic tip (Wessels 1993) is not known.

Mannitol-specialized bacteria have been found in association with *S. bovinus* mycelia growing in forest soil (Timonen et al. 1998) and this compound is, coincidentally, readily exchanged through the *S. bovinus* membrane in vitro. This correlation is worthy of further study. Bacterial activity supported by the exudate may feed the fungus with mobilized nutrients and, thereby, create a nutrient microloop from fungus, via bacteria, back to the symbiont (Unestam et al. 1999).

Calcium oxalate crystals were earlier found on the *S. bovinus* hyphae (Unestam and Sun 1995) but were formed in vitro only when calcium ions were added. Calcium is also provided by the hypha itself. Thus, hyphae of *Hysterangium setchellii* (on *Pseudotsuga menziesii*) may be loaded with crystals. In contrast, *Gautieria monticola,* another mycorrhizal fungal associate of Douglas-fir, produced large amounts of oxalic acid in the same soils without producing crystals to any comparable extent (Griffiths et al. 1994). Precipitation of calcium by exuded oxalate is considered a protection against harmful concentrations of the ion in the cell (Marschner 1995). This agrees with the low levels of Ca^{2+} in the exuded drops in our experiments.

Large numbers of calcium oxalate crystals are produced by ectomycorrhizal and VA mycorrhizal fungi (Graustein et al. 1977; Cromack et al. 1979; Jurinak et al. 1986; Unestam and Sun 1995) and oxalic acid was exuded by hyphae of *S. bovinus* in vitro. Oxalic acid may take part in the weathering of the mineral soil (Jurinak et al. 1986; Song and Huang 1988; Cromack and Caldwell 1992) and rocks (Unestam et al. 1999). It is toxic to fungal cells (Duschesne et al. 1989 a,b) and may need to be eliminated by many soil organisms. The brown-rot fungus *Coniophora puteana* can produce both acetic acid and oxalic acid, while *Serpula lacrimans* only produces oxalic acid (Bech-Andersen 1989). In our study, *S. bovinus* exuded both acids into the drops. Oxalic acid on the cell surface may remove hydrogen ions from the cytoplasm and thus adjust its pH. Furthermore, a high C/N ratio is likely to exist in these hyphae because absorbed nitrogen is continually withrawn by translocation to plant sinks. Some of the exuded metabolic carbon is, at the same time, removed by other microbes, while disposable compounds seem to accumulate outside the hyphae.

In summary, the results presented here, together with our conclusions, provide a unifying theoretical framework for further investigations of hyphal translocation, mycelial homeostasis and mycorrhizal interactions with other soil microorganisms. Studies of *uptake* by mycorrhizal mycelia abound but relatively little attention has been paid to the qualitative aspects of carbon flow to hyphal tips and the significance this may have for both exclusion of waste products and interactions with other soil microorganisms. Interactions between mycorrhizal mycelia and bacteria are attracting increasing attention but most studies have so far been largely descriptive (Frey et al. 1997; Frey-Klett et al. 1997; Nurmiaho-Lassila et al. 1997; Timonen et al. 1998). Improved understanding of the functional basis of such interactions will depend upon a better understanding of the regulation of mycorrhizal mycelial physiology and how this influences supply of different compounds to the mycelial-bacterial-soil interface.

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