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Review

Sugar for my honey: Carbohydrate partitioning in ectomycorrhizal symbiosis

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Abstract

Simple, readily utilizable carbohydrates, necessary for growth and maintenance of large numbers of microbes are rare in forest soils. Among other types of mutualistic interactions, the formation of ectomycorrhizas, a symbiosis between tree roots and certain soil fungi, is a way to overcome nutrient and carbohydrate limitations typical for many forest ecosystems. Ectomycorrhiza formation is typical for trees in boreal and temperate forests of the northern hemisphere and alpine regions world-wide. The main function of this symbiosis is the exchange of fungus-derived nutrients for plant-derived carbohydrates, enabling the colonization of mineral nutrient-poor environments.

In ectomycorrhizal symbiosis up to 1/3 of plant photoassimilates could be transferred toward the fungal partner. The creation of such a strong sink is directly related to the efficiency of fungal hexose uptake at the plant/fungus interface, a modulated fungal carbohydrate metabolism in the ectomycorrhiza, and the export of carbohydrates towards soil growing hyphae.

However, not only the fungus but also the plant partner increase its expression of hexose importer genes at the plant/fungus interface. This increase in hexose uptake capacity of plant roots in combination with an increase in photosynthesis may explain how the plant deals with the growing fungal carbohydrate demand in symbiosis and how it can restrict this loss of carbohydrates under certain conditions to avoid fungal parasitism.

Keywords: Sugar regulation; Ectomycorrhiza; *Amanita*; Poplar; Gene expression

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Abbreviations: ECM, ectomycorrhizal; TC, tentative coding unit; PAL, phenylalanine ammonium lyase; UTR, untranslated region.

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1. Introduction

Due to their litter and humus layers, forest soils are rather rich in complex carbohydrates (e.g., cellulose and lignin). These carbohydrates are only degradable by highly specialized microorganisms and destruction is a rather slow process. As a consequence, simple, readily utilizable carbohydrates that are necessary for the growth of large numbers of microbes (including most ectomycorrhizal fungi) are quite rare (Wainwright, 1993). In contrast to forest soils, exudates of living plants are rich in simple carbohydrates. Therefore, the association with plant fine roots allows a privileged carbohydrate access for microbes.

Forest soils are not only difficult for microbes but also for trees, since a large portion of nitrogen and phosphorus is stored in organic form in the litter layer. Because forest trees (in contrast to microorganisms) have only a limited capability to mobilize organic nutrients, their nutrition depends on microbial activity.

Among other types of mutualistic interactions, ectomycorrhizal symbiosis is one way to overcome nutritional and carbohydrate limitations faced by trees and certain soil fungi in forest ecosystems.

In boreal and temperate forests of the northern hemisphere and alpine regions world-wide ectomycorrhizas are the dominating type of mycorrhizal symbiosis among trees. Ectomycorrhizal host trees include members of the genus Pinaceae, Fagaceae, Betulaceae, Myrtaceae, Rosaceae, Salicaceae, Cistaceae and Dipterocarpaceae (Read, 1991, 1993; Smith and Read, 1997). While only a relatively small number of seed plants (about 2000 species) are ectomycorrhizal, their dominance in forests and their value as a timber source make them of global importance.

The fungal partners in ectomycorrhizas comprise the most advanced groups of Basidiomycetes, such as Boletaceae, Corticiaceae, Cortinariaceae, Tricholomataceae, Russulaceae and Thelephoraceae, a few Asco- (e.g. Elaphomycetaceae and Geneaceae) and Zygomycetes, adding up to about 5000–6000 species (Molina et al., 1992). The capability to participate in ectomycorrhizal symbiosis has evolved repeatedly from saprotrophic precursors and is not the result of a single ancestor (Hibbett et al., 2000), explaining the large (systematic) diversity of ectomycorrhizal fungi.

2. The fungal partner

2.1. The ectomycorrhizal fungal colony

A profound aspect of the ectomycorrhizal and wood decaying fungal lifestyle is the formation of large colonies. In contrast to fast growing, saprophytic fungi different parts of the ectomycorrhizal colony remain interconnected by living hyphae, and extensive nutrient and metabolite exchange takes place.

Soil growing hyphae that explore the litter for nutrients constitute large parts of the ectomycorrhizal fungal colony. To interconnect different parts of a colony (such as mycorrhizas and fruiting bodies), hyphal aggregates and cords or rhizomorphs are formed (Cairney et al., 1991), which enable the exchange of nutrients and carbohydrates over long distances.

When fungal hyphae recognize an emerging fine root of a compatible plant partner, they direct their growth towards it (Martin et al., 2001) and colonize the root surface, (often) forming a sheath or mantle of hyphae, which encloses the root, and isolates it from the surrounding soil (Blasius et al., 1986). Root hairs, which are normally formed by rhizodermal cells, are suppressed by ectomycorrhiza formation.

After or parallel to sheath formation, fungal hyphae grow inside the infected fine root, forming highly branched structures in the apoplast of the rhizodermis, or in the case of gymnosperms also in the root cortex. This so-called Hartig net creates a large surface area between both partners (Kottke and Oberwinkler, 1987).

Both fungal networks of ectomycorrhizas (fungal sheath and Hartig net) have different functions (Harley and Smith, 1983; Kottke and Oberwinkler, 1987; Smith and Read, 1997). The Hartig net, which serves as an interface between plant and fungus, is adapted to the exchange of plant-derived carbohydrates for fungus-derived nutrients. The function of the fungal sheath is that of an intermediate storage for (a) nutrients that are delivered by soil growing hyphae and are intended for the Hartig net and (b) carbohydrates that are taken up by hyphae of the Hartig net and are designated for transport towards the soil growing mycelium.

2.2. Fungal carbohydrate support in symbiosis

Due to the low content in readily useable carbohydrates in the soil, ectomycorrhizal fungi are ecologically dependent on a continuous carbon support by the plant during the growth season (Harley and Smith, 1983; Smith and Read, 1997; Leake et al., 2001). One of the first attempts to assay carbon flow in a mycorrhizal plant was performed by Melin and Nilsson (1957). They could show that feeding (^{14}C) CO_2 to leaves resulted in the appearance of labeled carbon in the hyphal mantle within one day. These results were confirmed by a number of researchers with different experimental systems later on (e.g. Lewis and Harley, 1965a,b; Cairney et al., 1989; Leake et al., 2001; Wu et al., 2002).

Potential carbon compounds delivered by the plant partner in symbiosis are soluble sugars, carboxylic acids and amino acids. Plant cell wall compounds like pectin, hemicellulose, cellulose, or proteins have also been under discussion (for reviews see Smith et al., 1969; Harley and Smith, 1983; Smith and Read, 1997). However, owing to the huge rates of carbon consumption by the fungal partner (up to 30% of the net photosynthesis rate; Söderström, 1992) cell wall compounds are not likely to constitute a major carbon source. And even though there are indications that some ectomycorrhizal fungi have some cellulolytic activity, the degradation rate is too slow to meet the fungal carbohydrate demand (Trojanowski et al., 1984; Haselwandter et al., 1990; Entry et al., 1991). Thus, soluble sugars and organic acids and are the most likely candidates.

When grown under axenic conditions (which does not necessarily reflect the situation in ectomycorrhizas), the majority of investigated ectomycorrhizal fungi investigated so far grow best on simple sugars like glucose and fructose (Palmer and Hacskaylo, 1970; Salzer and Hager, 1991).

The capability of ectomycorrhizal fungi to utilize sucrose (the major transport form of carbohydrates in many plants) is discussed controversially. While there are some indications in literature that some fungi might have sucrolytic activity, others clearly do not (Willard et al., 1987; Salzer and Hager, 1991; Schaeffer et al., 1995). It is thus commonly accepted that sucrose, which is excreted by plant root cells into the common apoplast of the plant/fungus interface, is hydrolyzed by a plant-derived invertase in ectomycorrhizal symbiosis (Lewis and Harley, 1965a; Salzer and Hager, 1991; Rieger et al., 1992; Hampp and Schaeffer, 1995; Nehls, 2004). The lack of an invertase in (at least many) ectomycorrhizal fungi is a profound difference to phytopathogenic (e.g. *Pyrenopeziza brassicae*, Walters et al., 1996, *Sporisorium reilianum*, Bhaskaran and Smith, 1993, *Uromyces fabae*, Voegele et al., 2001) but also ericoid mycorrhizal fungi (e.g. *Hymenoscyphus ericae*, Straker et al., 1992; Hughes and Mitchell, 1996). Since it makes the fungus dependent on the plant partner, lack of invertase might partially explain the low plant defense response faced by ectomycorrhizal fungi compared to pathogenic interactions (Smith and Read, 1997).

The driving force for carbon allocation in vascular plants is consumption at the sink site. Mycorrhizas attract carbohydrates much more efficiently than non-mycorrhizal fine roots (Bevege et al., 1975; Cairney et al., 1989), indicating a strong sink strength for fungal hyphae in symbiosis. Prerequisites for fungal sink formation are the ability of Hartig net hyphae to take up carbohydrates fast from the common apoplast and to quickly convert them into fungal metabolites, thereby maintaining a steep gradient between the apoplast and the fungal cytosol.

2.2.1. Monosaccharide uptake by fungal hyphae

While yeast contains more than 20 functional sugar transporters (Boles and Hollenberg, 1997), only two hexose transporter genes from *Amanita muscaria* (*AmMst1*, Nehls et al., 1998; *AmMst2*, Nehls, 2004) and one hexose transporter gene from *Tuber borchii* (Agostini and Stocchi, pers. comm.) have been investigated from ectomycorrhizal fungi so far. However, the currently sequenced genome of *Laccaria bicolor* (Martin et al., 2004) contains 15 putative MST genes (Nehls, unpublished), indicating that there is still a large gap in our knowledge about hexose import into ectomycorrhizal fungi.

In *A. muscaria*, hexose transporter gene expression is up-regulated by a threshold response mechanism, which depends on the extracellular monosaccharide concentration (Nehls et al., 1998, 2001b). In hyphae grown in the presence of hexose concentrations below 2 mM, *AmMst1* is expressed at a basal level, while higher concentrations trigger an at least sixfold increase in transcript levels. Since increased monosaccharide transporter gene expression is a slow process, it has been interpreted as an adaptation process to elevated hexose concentrations usually found only at the plant/fungus interface (Nehls, 2004).

When expressed in yeast, *AmMst1* revealed K_M values of 0.46 mM for glucose and 4.2 mM for fructose, indicating a strong preference for glucose (Wiese et al., 2000). *A. muscaria* hyphae also strongly favored glucose over fructose, even in the presence of excess fructose (20 mM vs. 1 mM; Nehls et al., 2001b). Similar preferences for glucose uptake have likewise been observed for the ectomycorrhizal ascomycete *Cenococcum geophilum* (Stülten et al., 1995) and other basidiomycete ectomycorrhizal fungi (Salzer and Hager, 1993; Smith and Read, 1997), indicating that a preference for glucose might be common to ectomycorrhizal fungi.

Like those of ectomycorrhizal fungi, most plant monosaccharide transporters investigated so far also show a clear preference for glucose (Büttner and Sauer, 2000). Since sucrose is hydrolyzed into equimolar amounts of glucose and fructose, an increased apoplastic fructose concentration (>2 mM) would be the result of a preferential glucose uptake occurring at the Hartig net. Because fructose uptake by fungal hyphae is efficient at low apoplastic glucose concentrations, fructose withdrawal from the apoplast presumably takes place within the innermost one or two layers of the fungal sheath (Wiese et al., 2000; Nehls et al., 2001a; Nehls, 2004), resulting in a steep apoplastic hexose gradient between hyphae of the Hartig net and those of the fungal sheath.

2.2.2. Fungal carbohydrate metabolism

For the creation of a strong carbon sink in symbiosis, monosaccharides taken up by hyphae of the Hartig net have to be converted into fungal metabolites quickly, either by (a) increased carbon flux through glycolysis or (b) by the generation of fungal storage compounds.

Like in other organisms, the enzyme phosphofructokinase performs the rate-limiting step in ectomycorrhizal fungal glycolysis (Kowallik et al., 1998). In *A. muscaria* this enzyme is activated by fructose 2,6-bisphosphate

(F26BP; Ka about 30 nM; Schaeffer et al., 1996), which has similar properties to the corresponding enzyme in yeast or animal cells, but differs from plant phosphofructokinases. It has been shown that *A. muscaria* mycelia grown in the presence of high hexose concentrations as well as the symbiotic mycelium have increased amounts of F26BP (Schaeffer et al., 1996). This may indicate increased rates of gycolysis in hyphae under elevated hexose supply, such as hyphae of the Hartig net (Hampp et al., 1999; Nehls et al., 2001a).

When grown in the presence of a rich carbon source, ectomycorrhizal fungi produce a series of fungus-specific sugars and sugar alcohols (Martin et al., 1985, 1987, 1988, 1998). Two different pools of storage carbohydrates can be distinguished in ectomycorrhizal fungi: short chain carbohydrates (trehalose) or polyols (mannitol, arabitol, erythritol), and the long chain carbohydrate glycogen.

Growth of *A. muscaria* in axenic culture on glucose as a carbon source resulted in an increase in trehalose content over time. However, when the external glucose concentration fell below 4 mM, the trehalose content was continuously depleted. In contrast, the glycogen content was stable during the whole period of investigation (Wallenda, 1996). Longitudinal dissection of single ectomycorrhizas showed that in the presumably most intense interaction zone the fungus-specific disaccharide trehalose dominates, indicating a conversion of glucose into this storage compound in functional mycorrhizas (Rieger et al., 1992). In *Lactarius* the glycogen content was high during winter, declined until summer (strong fungal propagation), and was restored during autumn (Genet et al., 2000). These data together indicate, that trehalose and polyols act as short term storage compounds, which undergo high rates of fluctuation, while glycogen is the long term storage carbohydrate of hyphae, which is only mobilized when the short term pools are empty.

Because ectomycorrhizal fungi commonly form an extensive external mycelium, and soil growing hyphae depend on carbohydrate support by mycorrhizas (Leake et al., 2001), long-distance transport of carbon is of great importance for fungal physiology. In *Paxillus involutus* ectomycorrhizas, glycogen particles were observed in the Hartig net and the inner and outer hyphal layers of the fungal sheath (Jordy et al., 1998). However, since glycogen is stored in the cytoplasm as large, non-mobile granules it is rather likely, that glycogen (similar to starch in plants) is not the long-distance carbohydrate transport form in fungi. In contrast, polyols and trehalose are present in large quantities in fungal hyphae and are (like sucrose in plants) good candidates for long-distance transport carbohydrates.

In summary, efficient glucose uptake (triggered by enhanced monosaccharide transporter gene expression) in combination with modulated fungal carbohydrate metabolism and carbohydrate removal towards other parts of the fungal colony (e.g. soil growing mycelium, Leake et al., 2001) may explain how a strong fungal carbohydrate sink is generated in symbiosis.

2.3. Carbohydrates as signal for the regulation of fungal physiology in ectomycorrhizas

"Metabolic zonation" and "physiological heterogeneity" have been discussed as important concepts for a functional understanding of ectomycorrhizal symbiosis (Cairney and Burke, 1996; Timonen and Sen, 1998). Differences in the apoplastic hexose concentration at the Hartig net vs. fungal sheath could be a signal that might regulate fungal physiological heterogeneity in ectomycorrhizas (Nehls et al., 2001a; Nehls, 2004).

Sugar-dependent gene expression in functional ectomycorrhizas was investigated in *A. muscaria* using hexose importer genes (*AmMst1*, Nehls et al., 1998 and *AmMst2*, Nehls, 2004) and a phenylalanine ammonium lyase (AmPAL, Nehls et al., 1999), which are diametrically regulated. PAL is a key enzyme of secondary metabolism and ECM-forming fungi have been reported to use phenolic compounds for protection against bacterial and fungal attacks (Marx, 1969; Chakravarty and Unestam, 1987; Garbaye, 1991).

In axenic culture, the expression of these genes was regulated by a threshold response mechanism depending on the extracellular monosaccharide concentration. Glucose concentrations of up to 2 mM resulted in a basal expression of both hexose transporter genes, while the PAL transcript level was maximal (Nehls et al., 2001b). At higher hexose concentrations a strong increase in sugar transporter gene expression occurred, while PAL transcripts were barely detectable.

In functional ectomycorrhizas, AmPAL was only detectable in hyphae of the fungal sheath, while elevated hexose transporter gene expression was exclusively observed in hyphae of the Hartig net (Nehls et al., 2001b). Owing to the opposing (sugar dependent) regulation of these genes in both hyphal networks, hexose concentrations above 2 mM can be expected in the apoplast of the Hartig net while lower concentrations are supposed to be present in hyphae of the fungal sheath (Nehls et al., 2001a).

Different external sugar concentrations, however, also trigger the expression of a number of other *Amanita* genes. As a result of an EST project using functional poplar ectomycorrhizal roots, 70 bp long oligomers from a total of 600 partially sequenced tentative fungal genes were spotted onto glass slides as part of the Pt2.4kOLI1 microarray (see Küster et al., this issue). These slides were hybridized with Cy-labeled first strand cDNA obtained from RNA of hyphae grown under axenic conditions either with 1 mM or 25 mM glucose as carbon source (Nehls, unpublished). Fig. 1 shows the differences in the transcript levels of the investigated genes with respect to fungal carbohydrate nutrition. In the presence of 25 mM glucose, 101 out of 600 investigated genes were at least 1.5-fold up- and 110 were down-regulated (Nehls, unpublished). The fact that one third of the genes were affected by glucose addition reveals the great importance of carbohydrate nutrition for fungal physiology. The difference in the

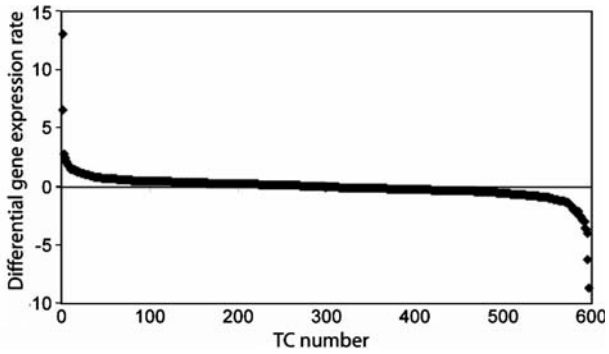

Fig. 1. Impact of the external glucose concentration on the expression of *A. muscaria* genes. Fungal mycelia were grown in the presence of 1 mM or 25 mM glucose as sole carbon source. Total RNA was extracted and aliquots of about 15 μg were used for cDNA synthesis (SuperScript Indirect cDNA Kit, Invitrogen, Carlsbad, CA, USA) coupled to indirect labeling with fluorescence dyes (cyanine 3 or cyanine 5, Amersham Biosciences, Little Chalfont, UK) as described in Küster et al. (2004). Equal amounts of first strand cDNA obtained from both fungal treatments (labeled with different dyes) were mixed and used for the hybridization of microarrays containing 70 bp long oligomers of 600 *A. muscaria* tentative coding units (TCs) as described by Küster et al. (2004). After separate detection of the fluorescence intensity for each fluorescence dye followed by Lowess normalization using the program package EMMA software (Dondrup et al., 2003; see Küster et al. this issue), the fluorescence intensity ratio was calculated for each TC. Positive values (*p*-value 0.05) represent TCs that are expressed higher in fungal hyphae grown at 1 mM glucose, negative values represent TCs that are expressed higher in fungal hyphae grown at 25 mM glucose. The TCs are put in order according to the observed differences in gene expression.

apoplastic hexose concentration in both fungal networks of ectomycorrhizas could thus be a key regulator for the adaptation of fungal hyphae to their different physiological functions in symbiosis.

In yeast and filamentous ascomycetes the external sugar concentration is sensed by monosaccharide transporter-like proteins (RGT2 and SNF3 in yeast, RCO3 in *Neurospora crassa*). These proteins regulate sugar-dependent gene induction, which is the first step in a signaling cascade (Celenza et al., 1988; Özcan et al., 1996; Madi et al., 1997). In addition to external sugar sensors, monosaccharide-dependent gene repression is regulated via a hexokinase-dependent signaling pathway (Ronne, 1995; Gancedo, 1998). The molecular mechanism of signal initiation is still unclear, but hexokinase (in yeast mainly Hex2) starts the signal in response to carbon-flux through the enzyme.

In *A. muscaria* AmPAL expression could not only be repressed by glucose but also by its analog 2-desoxy-glucose (which is phosphorylated by hexokinase), while 3-*O*-methyl-glucose (which is not phosphorylated by hexokinase) had no impact on gene expression (Nehls et al., 1999, 2001a). Thus, as in yeast, hexokinase obviously acts as a regulator of sugar-dependent gene repression in *A. muscaria*.

However, in contrast to ascomycetes, the sugar-dependent up-regulation of *Amanita* monosaccharide transporter genes seems to be regulated by an internal sensor (Nehls et al., 1998, 2001b). Sugar analogs that are imported into *A. muscaria* hyphae (3-*O*-methyl-glucose) and phosphorylated by hexokinase (2-desoxy-glucose) do not trigger up-regulation of *AmMst1* in the way that glucose does (Nehls et al., 1998; Wiese et al., 2000). This indicates, that a signal beyond hexokinase activity either in glycolysis or storage carbohydrate biosynthesis (Nehls et al., 2001a; Nehls, 2004) is responsible for sugar-dependent induction of gene expression.

3. The plant partner

An essential basis for mycorrhizal functioning is the fungal sugar supply by the host plant. McDowell et al. (2001) reported that carbon allocation to fine roots and mycorrhizas made up 47–59% of the total below ground carbon allocation, and several authors estimated that up to 30% of the net photosynthesis rate is necessary for fungal support in symbiosis (Söderström and Read, 1987; Söderström, 1992; Jones and Darrah, 1996; Farrar and Jones, 2000). In response to the huge carbohydrate drain in symbiosis, the plant can (a) increase its photosynthetic efficiency and (b) control carbohydrate loss toward the fungus to avoid fungal parasitism.

3.1. Enhanced photosynthetic efficiency of mycorrhized plants

There is ample evidence that assimilate allocation toward the root due to mycorrhization can upregulate the net photosynthesis rate of the host plant. As a response to mycorrhization Vodnik and Gogala (1994) found increased chlorophyll and carotenoid contents in needles of spruce seedlings mycorrhized with *Laccaria laccata*, *Pisolithus tinctorius* and *Lactarius piperatus*. Mycorrhized *Castanea sativa* plants have decreased respiration rates, resulting in a lower CO_2 compensation point and an increased amount of ribulosebisphosphate carboxylase (Martins et al., 1997). Furthermore, gas exchange measurements revealed an increased CO_2 fixation rate for mycorrhized Norway spruce and aspen plants (Friedrich, 1998; Loewe et al., 2000). Beside this, indirect evidence for an ectomycorrhiza caused increase in host plant photosynthesis exists. Lamhamedi et al. (1994) demonstrated that the removal of *L. bicolor* fruitbodies (which form a huge carbon sink) resulted in a rapid decrease in net photosynthesis of the host plants.

Thus, plant photosynthesis is enhanced in ectomycorrhizal symbiosis to meet the increased carbohydrate demand of the additional (fungal) carbon sink.

3.2. Control of carbohydrate drain by the host plant

To avoid fungal parasitism, the host plant must control the carbohydrate efflux in symbiosis. Regulation of the carbohydrate drain from the plant towards the fungus can occur at several levels. This includes (i) the control of

sucrose export into the apoplast (a still unknown mechanism), (ii) sucrose hydrolysis by plant-derived invertases, and (iii) competition between root cells an fungal hyphae for hexoses from the apoplast of the Hartig net.

3.2.1. Sucrose hydrolysis by plant-derived invertases

In contrast to parasitic biotrophic interactions, apoplastic sucrose hydrolysis by plant-derived cell wall acid invertases in ectomycorrhizal symbiosis is a prerequisite for fungal carbohydrate support (Salzer and Hager, 1993; Schaeffer et al., 1995). While acid invertase activity seemed not to be a rate-limiting step in Norway spruce (unaffected by mycorrhiza formation; Schaeffer et al., 1995), enzyme activity was strongly increased in *Betula* (Wright et al., 2000). This indicates that enzyme activity might be a checkpoint for fungal carbohydrate support in some plants.

3.2.2. Competition between root cells an fungal hyphae for hexoses

Based on their protein sequence, plant hexose transporters can be divided into four different subgroups (Fig. 2). Always one *Populus tremula* × *tremuloides* member of each of the subgroups I, II and III was expressed in fine roots (Grunze et al., 2004). The spruce transporter *PaMST1* that is also expressed in fine roots (Nehls et al., 2000) belongs to subgroup IV, in which no *P. tremula* × *tremuloides* homolog has been identified so far.

The impact of ectomycorrhiza formation on monosaccharide transporter genes expressed in tree roots has been investigated for one *Picea abies* gene (Nehls et al., 2000), two *Betula pendula* genes (Wright et al., 2000) and three *P. tremula* × *tremuloides* genes (Grunze et al., 2004; four additionally identified poplar transporter genes are not expressed in fine roots at all). For these experiments, four to five-month old plants were grown in a Petri dish system (poplar, spruce) or in flasks (birch). Compared to non-mycorrhized fine roots, the expression of *BpHEX1* of birch and its polar homolog *PttMST1.2* (subgroup I), *PttMST2.1* (poplar, subgroup II) and *PaMST1* (spruce, subgroup IV) was suppressed upon ectomycorrhiza formation. While the expression of *BpHEX2*, the second investigated *Betula* gene, was also repressed, the transcript level of its closest poplar homolog (*PttMST3.1*, subgroup III) was increased strongly (12-fold) in mycorrhizas.

But can this increase in *PttMST3.1* expression make up for the loss in expression of the other two poplar monosaccharide transporters *PttMST1.2* and *PttMST2.1*? Considering that in non-mycorrhized fine roots *PttMST3.1* was expressed at least 10 times higher than *PttMST1.2* and

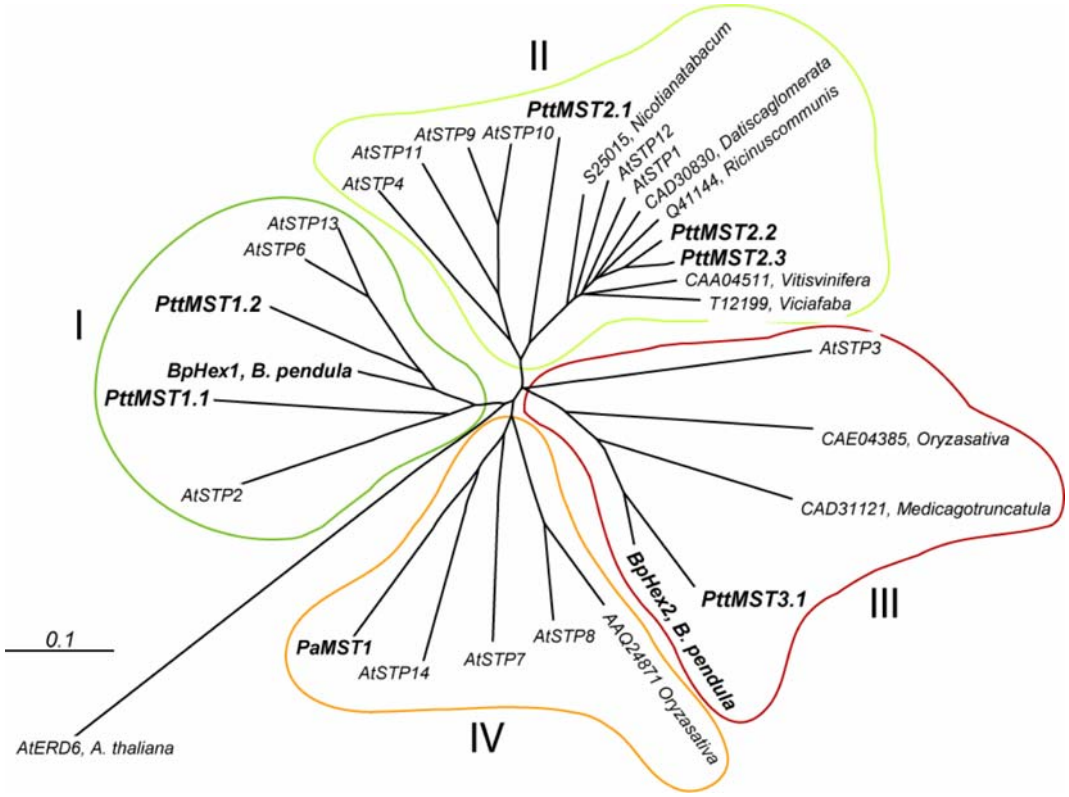

Fig. 2. Dendrogram of the alignment of the deduced protein sequences of *P. tremula* × *tremuloides* and other known plant monosaccharide transporters. All known *P. tremula* × *tremuloides*, birch, Norway spruce and *Arabidopsis* transporters as well as other selected plant hexose transporter proteins available in public databases were analyzed using ClustalW (Thompson et al., 1994) and using the *Arabidopsis* AtERD6 protein as an outgroup. The calculated dendrogram was visualized using the computer program TreeView (taxonomy.zoology.gla.ac.uk/rod.rod.html).

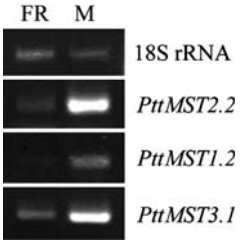

Fig. 3. Enhanced expression of poplar hexose transporter genes in ectomycorrhizal symbiosis. Total RNA was isolated from non-mycorrhizal fine (FR) roots as well as mycorrhizas (M) of two-year-old poplars grown in the field. Aliquots of about 0.4 μg were DNAse I treated and used for first-strand cDNA synthesis. The amount of first-strand cDNA used for quantitative PCR was calibrated using 18S rRNA (Grunze et al., 2004) and actin gene (Langer et al., 2002, data not shown) specific primers. PCRs were performed with specific primers from the 3′-UTRs of the hexose transporter genes.

PttMST2.1, the observed 12-fold increase in *PttMST3.1* expression strongly overcompensates the about 50% loss in transcript levels of *PttMST1.2* and *PttMST2.1* (Grunze et al., 2004). Therefore it can be concluded, that in poplar mycorrhizas the hexose uptake capacity of fine roots is strongly enhanced during symbiosis.

Why does ectomycorrhiza formation seem to have different effects on hexose transporter gene expression in spruce and birch compared to poplar?

Firstly, in the genomes of *Arabidopsis* and *P. trichocarpa* 14 and 23 (Nehls, unpublished) different potential hexose transporter genes, respectively, could be distinguished, indicating that the number of hexose transporter genes expressed in roots of spruce and birch is probably larger. Secondly, in contrast to the results obtained with poplar mycorrhizas from a Petri dish system (Grunze et al., 2004), the expression of all three analysed poplar monosaccharide transporters, *PttMST3.1*, *PttMST1.2* and *PttMST2.1*, was strongly increased in mycorrhizas obtained from three-year-old poplar plants grown under field conditions (Fig. 3, Willmann, unpublished).

The fact that the presence of more hexose transporter genes can be expected in Norway spruce and birch, and the results with poplar indicating that tree age can strongly affect gene expression (experiments with spruce and birch were performed with rather young plants), may explain why hexose transporter gene expression was found to be reduced by ectomycorrhiza formation in birch and spruce but enhanced in poplar.

The induction of plant cell wall invertase activity (birch, Wright et al., 2000; poplar, Nehls, unpublished) as well as poplar hexose importer gene expression (Grunze et al., 2004, Willmann, unpublished) in ectomycorrhizal symbiosis resembles that of pathogenic interactions (e.g. Bhaskaran and Smith, 1993; Walters et al., 1996; Hahn and Mendgen, 2001) observed in plant leaves. Such mechanisms that could restrict apoplastic carbohydrate concentrations are thus presumably a general mechanism in plant/microbe interactions.

4. Linking fungal carbohydrate and plant nutrient support in ectomycorrhizal symbiosis

In *A. muscaria*/*P. tremula* × *tremuloides* ectomycorrhizas, expression of monosaccharide importer genes of both fungal and plant origin is strongly enhanced. As a consequence, intense competition for apoplastic hexoses at the plant/fungus interface can be supposed. This raises the question of how the observed net transfer of large amounts of carbohydrates toward the fungus can occur under these conditions.

PttMST3.1 (which is expressed at least 10-times stronger in non-mycorrhizal poplar fine roots than any other poplar hexose importer) contains potential phosphorylation sites (Grunze, unpublished). Furthermore, although *PttMST3.1* was correctly transcribed, neither heterologous expression in yeast nor *Xenopus laevis* oocytes was successful (Grunze et al., 2004). Thus, the import of hexoses by *PttMST3.1* might be prevented prior to posttranslational activation (e.g. phosphorylation). It can thus be speculated that poplar plants increase their potential carbohydrate uptake capacity (as indicated by enhanced gene expression), but do not use it extensively as long as the fungus provides sufficient nutrients (Fig. 4). Regulation of the sugar uptake capacity by posttranslational mechanisms, such as phosphorylation, would allow a much faster response to alterations in the environment (e.g. reduced fungal nutrient support).

To test this hypothesis, transgenic poplar plants were generated, that overexpress an additional hexose importer gene under conditions that cannot be controlled by the plant (Nehls, unpublished). These transgenic plants reveal a reduced mycorrhizal infection capability and an abnormal termination of the symbiosis which has never been observed for non-transgenic poplars under laboratory conditions.

Also in literature some indications exist, that the amount of photoassimilates transferred to the fungal partner depends on fungal nutrient delivery. *Populus* and *Pinus sylvestris* plants were double-labelled in pulse chase experiments with (^{33}P) inorganic phosphate (Pi) and (^{14}C) CO_2 in darkness or under illumination. X-ray microanalysis and microautoradiography, showed that shading (less carbon allocation to the fungus) caused decreased Pi absorption by the mycobiont and, as a consequence, a reduced transfer to the host plant (Bücking and Heyser, 2001, 2003).

However, the extent to which the host has to pay for nutrient delivery depends on the fungal partner. Bidartondo et al. (2001) used combinations of *Pinus muricata* with different ectomycorrhizal fungi (*P. involutus*, *Suillus pungens*, Rhizopogon species) in microcosms to investigate how the availability of nutrients in the soil affects fungal carbon allocation. *P. involutus* produced the lowest biomass of ectomycorrhizal connections, but it consumed proportionally more carbon per connection than the other investigated fungi. Thus, certain fungi seem to be able to overcome (at least partially) the plant carbon drain control.

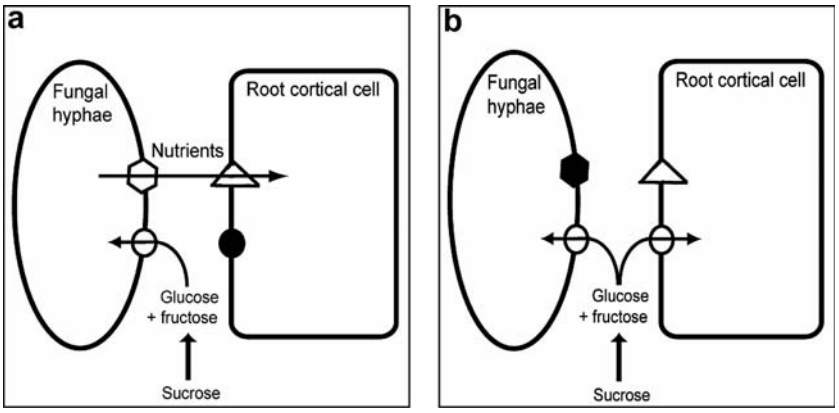

Fig. 4. Model for local coupling of plant nutrient and fungal carbohydrate support. Carbon and nutrient fluxes at the Hartig net are shown under conditions where the fungus is supporting its host plant with (a) sufficient or (b) insufficient nutrient amounts. Sucrose is thought to be hydrolyzed into hexoses by plant-derived acid invertases, and monosaccharides are taken up by monosaccharide importer (○). Nutrient exporters are represented by a hexagon and nutrient importers by a triangle. Inactive proteins are shown as filled, active proteins as open symbols.

In summary, there are several indications that fungal carbohydrate support by the host plant is linked to plant nutrition. This would allow the plant to control fungal carbohydrate support locally as well as at a whole plant level. However, since different ectomycorrhizal fungi seem to have varying capabilities to attract carbohydrates, mechanisms to bypass the supposed control mechanism must exist.

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