# Carbon transfer between plants via common mycorrhizal networks

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## Abstract

Evidence that shows the existence of interplant carbon transfer via common ecto- and arbuscular mycorrhizal networks is reviewed on basis of literature study. Although physiological and ecological investigations have provided evidence for some prerequisites for this kind of transfer, there is no direct evidence available. This may be caused by the used research methods that possibly underestimated the species specificity of the interaction between the mycorrhizal partners, or did not respect the importance of the plants' growth rates and the period of labeling with isotopes. This may be why the amounts of carbon found to be transported into the shoots of receiving plants have been very small in experiments where the transfer between arbuscular mycorrhizal networks was investigated.

## Introduction

Mycorrhizae are associations between plants and fungi that colonize the plant roots during periods of active plant growth. These associations have always been thought to be characterized by transport of carbon to the fungus and inorganic compounds (nutrients and water) to the plant. Mycorrhizae are very abundant among the higher plants (approximately 80-95% of all plant species associate with a mycorrhizal fungus species). Of the four groups of mycorrhizae the Orchidaeous and Ericaceous mycorrhizae are limited to species of Orchidaceae and Ericaceae families respectively. On the contrary, the other two groups, Vascular-Arbuscular mycorrhizae and Ectomycorrhizae, are not limited to a single plant family (e.g. Newman and Reddell, 1987; Peat and Fitter, 1993). This enables the possibility of one fungus individual connecting more plants of one species, but also of more species or even families (e.g. Newman, 1988). A network of intercellular hyphes (Hartig net) and a dense sheath of hyphae around the root distinguish Ectomycorrhizae from other groups. Arbuscular mycorrhizae consist of intercellular hyphae and intracellular fungal structures, the arbuscules and vesicles.

Since Francis and Read (1984) found indications for transport of carbon from one plant to another via mycelia, this possibility and the possible ecological implications of it have been investigated and discussed many times. There also is discussion about the way the existence should be proven.

In this paper I will investigate on the basis of literature study if the evidence for the existence of interplant nutrient transfer via common mycorrhizal networks is sufficient and whether the generally accepted theory (that only transport of carbon to the fungus and inorganic nutrients and water to the plant occurs) should be maintained or rejected. I leave Orchidaeous and Ericaceous mycorrhizae out because of the different nature of these symbioses.

I will give an abstract of the mechanisms that play a role in the transport of carbon and inorganic nutrients and how the carbon taken up by the roots is used by the plant. Then I will give some criteria for the evidence for interplant nutrient transfer via mycorrhiza and I will discuss the research done with respect to these criteria.

# Mechanisms of transport in common mycorrhizal networks

## *Transport in the interface*

There are two types of interface between the fungus and the host: inter- and intracellular. Both exist of the plasma membrane of the involved cells and the apoplasmatic space in between. The hyphes of intercellular interfaces grow between the cells of the root cortex, from which they are separated by the cell wall. Intracellular interfaces consist of invaginations of the host cell with a fungal structure (like an arbuscule or a vescle) growing inside. The fungus is not continuous with the protoplast of the host cell (Smith and Smith, 1990; Gianinazzi-Pearson *et al.*, 1991). The apoplasmatic space in the invaginations is partially separated from the other apoplasmatic space in the cortex and the soil by a collar consisting of loosely organized host membrane, around the basis of the fungal structure in the invagination (Smith and Smith, 1990; Gianinazzi-Pearson *et al.*, 1991). The ontogeny of the intracellular arbuscules coincides with the synthesis of the peri-arbuscular membrane (PAM), which is not as thick as the rest of the plasma membrane (Smith and Smith, 1990).

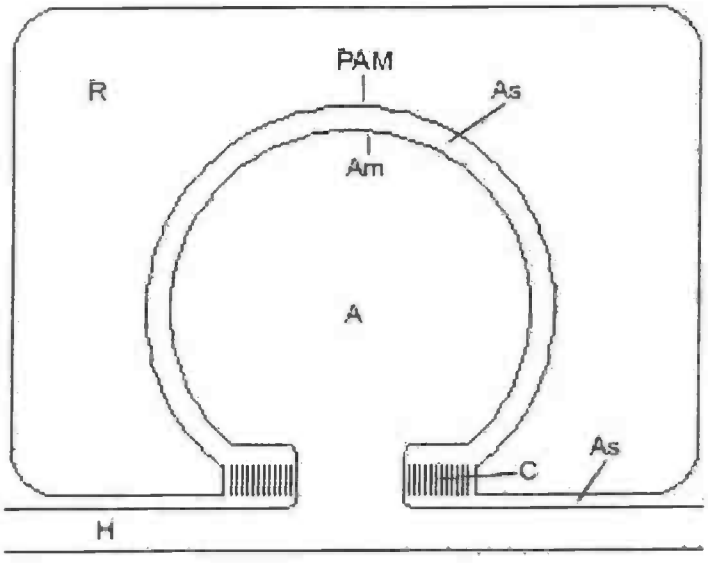


*Figure 1. Schematic representation of an arbuscular interface. A = arbuscule; R = root cell; H = hyphe; Am = arbuscular membrane; PAM = peri-arbuscular membrane; As = apoplasmatic space; C = collar of host membrane.*

Traditionally it is assumed that in these interfaces transport of nutrients is bi-directional: mineral nutrients to the plant and carbon to the fungus. Exudation of these substances is a passive process that is driven by concentration differences over the membrane and turgor (Smith and Smith, 1990; Jones and Darrah, 1992, 1996; Sun *et al.*, 1999). Uptake of nutrients from the apoplast is an active process that takes place under influence of $H^+$-ATP-ase in the plasma membranes of both fungus and plant (Marx *et al.*, 1982; Smith and Smith, 1990; Gianinazzi-Pearson *et al.*, 1991; Smith *et al.*, 1994). If an arbuscular fungus encounters a non-host plant, the fungus can penetrate the cortex of the root, but a fully functional symbiosis will not be formed. This can be concluded from the absence of

intracellular arbuscules (Giovannetti *et al.*, 1994) and the lack of $H^+$-ATP-ase activity in the membrane of infected cortex cells (Giovannetti and Sbrana, 1998).

Jones and Darrah (1992, 1996) have shown that maize exudates sugars and it can actively take up sugars from the rhizosphere against a concentration gradient. Sun *et al.* (1999) have shown that fungi exude water with dissolved solutes at the tips of growing hyphes, a property that has been known of plants for some time (e.g. Whittingham and Read, 1982; Jones and Darrah, 1992).

Both plant and fungus thus exude carbon into the apoplasmatic space of the interface and can both re-sorb solutes from it. It is probable that mixing of the exuded solutes occurs and that the plant will take up carbon that originates from the fungus. The uptake rates of both partners partially determine the net direction of the transport. The composition of the carbon compounds in the interface also determines how much each partner absorbs. Glucose is hydrolyzed into hexose by enzymes in the interface, which can be readily taken up and utilized by the fungus, but not by the plant (Smith and Smith, 1990) and fungi exude compounds which they cannot use. The plant takes up some of these compounds, like arabinose and ribose (Sun *et al.*, 1999).

### *Transfer through the fungus*

Transport of carbon compounds originating from photosynthesis occurs along a concentration gradient and by means of mass flow in direction of sinks (Brownlee *et al.*, 1983; Sun *et al.*, 1999), which can be growing parts of the mycelium, storage organs of the fungus, or receiving plants. Shadow increases the sink strength of receiving plants (Brownlee *et al.*, 1983; Jones en Darrah, 1992; Simard *et al.*, 1997). A possible explanation is that the receiving plant has to fulfil its carbon requirements by other means than photosynthesis. Water transport takes place along a potential gradient that originates from evaporation in the sink (mass flow). The volume can be sufficient for the receiving plant to foresee in its needs (Duddridge *et al.*, 1980; Brownlee *et al.*, 1983). Important is the buildup of turgor in the source by uptake of, amongst others, products of photosynthesis (Sun *et al.*, 1999). This transport through differentiated fungal cells is, with respect to the velocity and morphology, comparable with transport through xylem.

### *Use of carbon absorbed by the plant*

The use of the carbon taken up is dependent of the compound that is taken up. This has been investigated in plants without mycorrhizae (Farrar, 1985; Jones and Darrah, 1992, 1996). The compounds taken up by the plant can be used in metabolism, for the synthesis of new biomass and they can be stored as reserves. Farrar (1985) fumigated plants with labeled $CO_2$ and concluded after radiography that the compartmentalization of the assimilated label is the same as the compartmentalization of carbon taken up by the roots.

Plants use approximately 42% of the absorbed sugars for respiration (Farrar, 1985; Jones and Darrah, 1992). Possibly part of the label assimilated in the shoots originates from the air. This label could be brought into the air by, amongst others, respiration by the fungus, microorganisms in the soil or even by the plants' own roots. Jones and Darrah (1992) captured all $CO_2$ from the air by leading the air through two alkaline traps. Their results were comparable to others (Farrar, 1985), indicating that uptake by the roots was the major source of carbon in the plants.

The label that was assimilated into biomass was recovered in the growing parts of the plants. Jones and Darrah (1996) found that 89.9% of the label in the roots was assimilated into newly formed biomass at the root tips. According to their calculations and the results of Farrar (1985), in the root system approximately 30% of all carbon taken up is used in biomass production. The observed amounts of label transported to the shoots (as % of total uptake of label) were similar in these experiments: 0.17-0.2% $h^{-1}$ (Farrar, 1985; Jones and Darrah, 1992).

A large part of the absorbed sugars is temporarily stored in one of three dynamic pools: storage as starch or other polysaccharides, in the vacuole and in the apo- and cytoplasm. The starch pool is used up very slowly, the vacuole storage moderately and the apo- and cytoplasmic pool fast (halftime constants respectively 17, 5 and 0.5 hours) (Farrar, 1985). Jordy *et al.* (1998) investigated the location of several (insoluble) polysaccharides in the root tips and nearby hyphes during the first days of development of ectomycorrhizae. The ontogeny of the association involved accumulation of starch grains in the root cells and glycogen in fungal cells near the root tip and thickening of the root cell walls. After several days the accumulations and thickenings decreased and after eight days there was no accumulation of polysaccharides, or differentiation detectable anymore.

## Evidence for plant-to-plant transfer via common mycorrhizal networks

Which is the ideal method to show inevitably that products originating from photosynthesis are being transported from one plant to another by means of a common mycorrhizal network? One has to show that there is transport of solutes in the first place: the receiving plant has to take up solutes that originate from the source plant (Newman, 1988). In the second place it has to be assured that there is net transport and as third one has to show that (an important part of) the nutrients is transported through the fungus (Robinson and Fitter, 1999).

Many times researchers have thought they had shown inevitably that transport via common mycorrhizal networks occurs. This led to discussions time after time. Below I will give an overview of the (plant ecologically oriented) investigations and I will try to find out which of the experimental setups could meet the above criteria and whether the results of the experiments are to be kept as evidence.

### *Did transfer of carbon occur?*

Because it is, due to the intertwining structure of both partners, impossible to differentiate between fungus and plant in the analysis of root tissue, one must look for the carbon in the shoot of the plant. Some of the carbon that has been absorbed by the roots can be recovered in the shoot of the plant (o.a. Farrar, 1985; Jones and Darrah, 1992, 1996). Some of the carbon isotope can be fixated from the air. It is possible to separate the shoot from the soil by covering with Parafilm and capture the carbon in the air around the shoot in order to minimalize assimilation by photosynthesis (Jones and Darrah, 1992; Waters and Borowicz, 1994).

Duddridge *et al.* (1980) grew three seedlings of *Pinus sylvestris* (inoculated with *Suillus bovinus*) 142 days in peat. The fungus had colonized all peat in the containers after this period. Hereafter rhizomorphs were fed $^3H_2O$ from cups in the upper part or on the bottom of the containers. The $^3H_2O$ was recovered in the rhizomorphs, the roots (with attached hyphes) and the needles (especially the younger ones). It was not recovered in the peat below the cups or in a plant that was not associated with the fungus. This showed that both rhizomorphs and plants absorb $^3H_2O$. With another experiment with *P. sylvestris* and *S. bovinus* Brownlee *et al.* (1983) studied the transport of carbon from plants with established mycorrhizae to plants with developing mycorrhizae. Shoots of *P. sylvestris* with well developed mycorrhizae were exposed to labeled $CO_2$ and 24 hours after labeling the distribution of the label was examined using autoradiography. Brownlee *et al.* concluded from the thus obtained picture (figure 2) and the recovery of small amounts of label in the shoots of the unlabeled plants that carbon transfer between the plants had occurred. On the autoradiograph of Brownlee and coworkers the roots of the unlabeled plant (on the right) are visible as thin (unlabeled) lines in the middle of two labeled zones (figure 2.b.). This may be indicating that only minor amounts of carbon were absorbed, the largest part remaining in the fungus or the cortex of the roots.

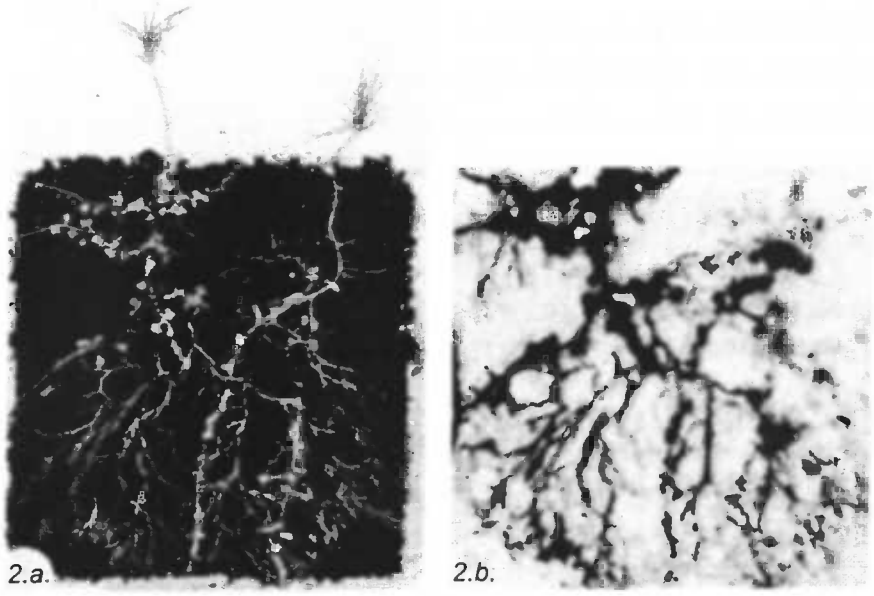


*Figure 2.a. Perspex container with infected seedlings of* Pinus sylvestris, *ten weeks after transfer to the peat. The seedling on the right hand side is the younger, recently infected. 2.b. Autoradiography of the container in 2.a., 24 hours after exposure of the older seedling to* $^{14}CO_2$ *(taken from Brownlee* et al., *1983).*

Francis and Read (1984) concluded that transfer of carbon occurred via the mycelium from the results of an experiment that was similar to that of Brownlee *et al.* (with *Plantago lanceolata* as donor and *Festuca ovina* as receptor, connected by *Glomus caledonium* though). The carbon is accumulated largely in previously existing vesicles outside the root and in hyphes that colonize young roots. Only a very small amount of label (depending on the amount of shadow applied to the plants 0.1-4% of the amount in the roots) was recovered in the shoots of unlabeled plants after labeling. The plants were exposed to the label for two days. Thereafter they were washed and the label was measured.

Grime *et al.* (1987) grew vegetation with and without mycorrhizae in microcosms for their well known experiment. 72 hours after labeling of donor plants (*Festuca ovina*) with $^{14}CO_2$ the shoots of the unlabeled mycorrizous plants contained a much larger amount of label (on average 45.6 times as much) than the non-mycorrizous plants.

Ek and coworkers investigated transfer of carbon between *Picea abies* and *Betula pendula* via *Scleroderma citrinum* and transfer of $^{15}N$-ammonium from the fungus to both plants (Ek *et al.*, 1996). The plants were grown under identical circumstances while each of both shoots was fumigated with air that contained either $^{12}CO_2$ or $^{13}CO_2$. The plants were harvested and the amount of label in the shoots and roots was determined after 72 hours. *B. pendula* received most nitrogen and *P. abies* received more carbon from *B. pendula* (which contained more label than *P. abies*) than vice versa.

The effect of mycorrhizae on transfer of $^{13}C$ in turfs of *Festuca ovina* was investigated by Graves *et al.* (1997). They fumigated half of each turf with labeled air. 41% of the label in infected donor plants was transferred to the unlabeled plants within a week, but none of the label was recovered in the shoots of the receiving plants. No label was transferred in the uninfected turfs.

Simard *et al.* (1997) investigated bi-directional and net transfer between *Betula papyrifera* and *Pseudotsuga menziesii*. Trees of the two species were labeled with either $^{13}CO_2$ or $^{14}CO_2$. After nine days the trees were harvested and separated into foliage, stems, coarse roots and fine roots, in which the concentrations of the two isotopes were determined. Bi-directional transfer between both tree species occurred in both years the experiment was carried out. Only in the second year net transfer to *P. menziesii* occurred. 13 and 45% of the transferred carbon was allocated to the shoots (for *P. menziesii* and *P. papyrifera* respectively).

Whether transferred carbon is taken up by the plant or remains in fungal structures has been investigated by Waters and Borowicz (1994) and Fitter *et al.* (1998) by clipping the aboveground parts of living plants. They assumed that carbon from the roots is necessary for re-growth of the clipped shoots. In neither experiment transfer of label to the re-grown shoots was found and it was concluded that the transferred carbon remains in fungal structures.

## *Has net transfer been found?*

The net transfer is interesting because it gives information about the benefits and costs of the nutrient transfer for the plants. The amount of transferred carbon is interesting from an ecological viewpoint to determine the influence of mycorrhizae on the competition between individual plants. Net transfer can be measured by using stable isotopes and calculating the diference between the amounts of isotope in donating and receiving plants. If one labels plants without knowing the amount of isotope naturally present in donor and receptor plants one cannot determine the magnitude of transfer.

Several researchers have shown transfer of carbon between plants using $^{14}C$, but they were unable to determine net transfer. This is caused by the fact that with $^{14}C$ it is impossible to determine net transfer because the specific activity of the source is unknown (Graves *et al.*, 1997).

Graves *et al.* (1997) and Fitter *et al.* (1998) have overcome this problem by fumigating half of the plants in their containers with $^{13}CO_2$ in equal concentration as the $^{12}CO_2$ that the rest of the plants received in the air. The ratio between these isotopes as they naturally occur in plants is known exactly. Application of the label should last at least a week in order to change this ratio enough to measure it (Graves *et al.*, 1997). Graves *et al.* investigated transfer of carbon between plants in turfs by fumigating one half of each turf with air containing $^{13}CO_2$. They found that 41% of the assimilated and to the root transported label was transferred to the unlabeled halves of the turfs, but they failed to recover label in the shoots of unlabeled plants. They concluded from this result that transfer of carbon to the fungus had occurred, but that it did not leave the fungus to the roots of unlabeled plants. Graves and coworkers found that in mycorrizous plants significantly more carbon was transferred to the roots than in non-micorrhizous plants (36 in stead of 10%). They attributed this to the age of the turves (>1 year), which accounts for the good development of the mycorrhizae. Interspecific transfer was <10%, but according to the authors that was due to the age of that association (several weeks).

Unfortunately part of the experimental setup of Fitter *et al.* (1998) failed to work. They didn't have results that give a clear picture about the carbon transfer between plants. Ek *et al.* (1996) did label with $^{13}C$, but they did not use the knowledge about the natural abundancy of this isotope and

they failed to measure net transport. 93% of the $^{13}C$ that they found in the mycelium originated from *B. pendula* and 94% of the $^{15}N$ from the mycelium was recovered in *B. pendula*.

### *Was the carbon transferred via the mycorrhizal network?*

Including one or more plants in the experimental setup that are not associated with the fungus can relatively easily show that transfer occurred via the fungus. One must also analyse those plants to find out if they have absorbed some of the label. These plants can be physically separated from the rhizosphere of the other plants in the experiment, but it is also possible to use plants that do not form mycorrhizae or mycorrhizae of another type. One has to assure that the other plants in the experiment are unable to associate with a fungus of the other mycorrhiza type. It is important to keep in one's mind that the fungus of the other type may absorb nutrients from the donor's rhizosphere.

It was not unequivocally shown that the water in the experiment by Duddridge *et al.* (1980) occurred via the fungus, but it was strongly indicated by the lack of label in the not-associated plant and the surrounding peat. Brownlee *et al.* (1983) showed clearly that the fungus was the major pathway for water transport by growing *P. sylvestris* with *S. bovinus* in peat or in petri dishes with a plastic barrier in the middle. Only the lower part of the peat, which contained hyphes but no roots, was fed with a nutrient solution. The few rhizomorphs that connected both parts of the peat transported enough solution for the plant (that grew in the upper part) to stay alive.

Francis and Read (1984) showed with autoradiographic pictures that the major part of the label in their experiment was transferred through the hyphes. On the pictures, taken after two days of labeling, the roots of the donor plant and the fungal structures are clearly visible whilst there is no label visible in the surrounding medium (figure 3).

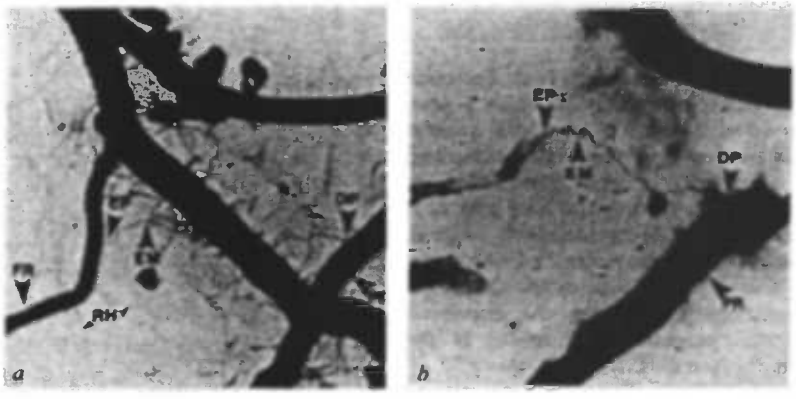


*Figure 3. a. Light-microscope image of an area showing roots of* Festuca ovina *(FR) and* Plantago lanceolata *(PR), connected by* Glomus caledonium. *b. Autoradiograph from the same area, after two days of exposure to label, showing that transfer of label had taken place between* P. lanceolata *(donor) and* F. ovina. *In both a. and b. the departure and entry points of the label have been marked (DP and EP respectively) (taken from Francis and Read, 1984).*

Grime *et al.* (1987) labeled *Festuca ovina* in their microcosm experiment and measured the amount of label in nearby plants 72 hours later. The mycorrizous plants received on average 45.6 times the amount of label as plants of the same species in microcosms without the mycorrhizae.

Plants of *Rumex acetosa*, which is non-mycorrizous by nature, did not absorb a greater amount of label when mycorrhizae were introduced in the turfs.

In the experiment by Simard *et al.* (1997) plants were included that are unable to form ectomycorrhizae, but arbuscular mycorrhizae in stead, to show that the major part of label was transferred through the hyphae. These controls absorbed small amounts of label, <1% and 18% of the total carbon transferred during respectively the first and second year of the experiment. Robinson and Fitter (1999) noted correctly that 18% is a substantial part, which causes rise of doubts about the patway of transport during that year.

Fitter *et al.* (1998) were hindered in their experiment by disfunctioning of the micro-pore septum they used to prevent growth of hyphae between two compartments in their experimental setup. Nevertheless they conclude that transfer of the label occurred through the hyphae because otherwise part of it would have been assimilated in the roots of unlabeled plants by photosynthesis.

## Discussion

From research on the physiology of mycorrhizae and the symbionts it is clear that when a fungus meets a compatible host plant, a tight symbiosis can evolve. The symbionts become so tightly interwoven that separation is not possible. This makes the analysis of assimilated label in root tissue virtually useless. The label has to be searched in the shoots of the plants, but translocation of absorbed carbon occurs at a slow rate. Transport through the fungus is a very fast process (Duddridge *et al.*, 1980), thanks to specialized rhizomorphs that enable the fungus to quickly allocate the nutrients necessary for growth of the mycelium to the places where they are needed most.

The results of the investigations on ectomycorrhizae (Duddridge *et al.*, 1980; Brownlee *et al.*, 1983; Ek *et al.*, 1996; Simard *et al.*, 1997) all led to the conclusion that transport occurred. Some of the assimilated label was recovered in the shoots and roots of the unlabeled plants, varying in the shoots from 0.2% in three days (Ek *et al.*, 1996) to 9.5% in nine days (Simard *et al.*, 1997). Only were Simard and coworkers able to determine net transport as a result of their experimental desing, using $^{13}C$. In the second year of their experiment net transport (2.7-9.5%) of carbon to *P. menziensii* occurred. Brownlee *et al.* (1983) and Simard *et al.* (1997) showed that the major part of the carbon transfer was via the fungus.

The experiments on arbuscular mycorrhizae (Francis and Read, 1984; Grime *et al.*, 1987; Graves *et al.*, 1997; Fitter *et al.*, 1998) have also shown transfer of carbon into and through the fungus. Francis and Read and Grime *et al.* recovered label in the shoots of receiving plants, indicating that transferred carbon is available to unlabeled plants. Graves *et al.* and Fitter *et al.* found no label in the shoots. Graves *et al.* found net transfer of carbon. That the transfer occurred via the fungus can be conluded from most of these experiments.

The results of physiological research indicate that transfer of carbon between plants connected by common mycorrhizal networks is possible. Ecological research aiming at showing actual transfer has not provided conclusive evidence. The results indicate that several criteria have been met. Below I discuss two of the causes that can be related to the used research methods, species specificity of the fungus-host interaction and carbon allocation in the receiving plant.

### *Species specificity of fungus-host combinations*

Several species of fungi were used in the investigations on one plant species: Francis and Read (1984) investigated transfer between *Plantago lanceolata* and *Festuca ovina* connected by *Glomus caledonium*, Fitter *et al.* (1998) investigated transfer between *P. lanceolata* and *Cynodon dactylon* connected by *Glomus mossae*. Although Fitter *et al.* had a longer period between labeling and measurement, they did not recover any label in the shoots of the unlabeled plants. They did find significant transfer of label into the fungus. The use of different fungal species may be a cause for the difference in the results.

The plant has generally a central position in the research on and considerations about the function of mycorrhizae in vegetations. Mostly, the fungus is implicitly presented as a functional tool in stead of a symbiont with an interest in the association. Richardson *et al.* (2000) wrote that the interaction between plants and fungi is not very species specific. The worldwide character of the fungal species' occurrences would create the situation for mycorrhizae to develop anywhere, except for ectomycorrhizae, because the Southern Hemisphere lacks enough host plants. The worldwide occurrence of fungal species can be concluded from the results of Morton and Bentivenga (1994), but they wrote about the occurrence of species types, not of individual species.

An inventory by Aziz *et al.* (1997) of the abundancy of arbuscular fungus species in the Everglades (VS) shows that not all species are evenly abundant. The relative abundance of *Glomus caledonium* and *G. mossae* was respectively 2 and 18%, over 51 sample points, in contrast to the abundance of *G. geosporum*, which was present in all samples. Of the investigated arbuscular mycorrhizae in the literature used for this paper the specific infections are based on *Glomus caledonium* and *G. mossae* (Francis and Read, 1984, Fitter *et al.*, 1998). The other arbuscular infections were initiated with wild, in nature infected plants of the same species as used in the experiments (Francis *et al.*, 1986; Grime *et al.*, 1987; Moora and Zobel, 1998), a mixture of spores (Graves *et al.*, 1997) or soil samples from the place where the plants originated (Waters and Borowicz, 1994).

Van der Heijden *et al.* (1998) varied the diversity of the fungal flora in an experiment with artificial grassland vegetation. The results show clearly that the relative biomass of different plant species depended on the occurrence of specific fungal species in the soil. This is a strong indication for a species-specific interaction between fungus and host.

For the fungus it is always positive to colonize a plant, because it can benefit from the carbohydrates exuded by the plant. It is not very disadvantageous for the fungus if the plant uses some of the exuded mineral nutrients (Wilkinson, 1997); the fungus is very well able to take up inorganic nutrients from the surrounding soil. Tolerance of infection by a non-compatible fungus is apparently advantageous for the plant; as long as the fungus does not make arbuscules the gain of inorganic nutrients is more than the small loss of carbon in the intercellular interfaces. If the fungus tries to make arbusculae the plant does not cooperate or the infected root cell dies to prevent large carbon loss.

I doubt if compatible plant-fungus combinations have been investigated in all research on arbuscular mycorrhizae. Successful infections have often been deducted from the percentage of the roots that was infected by the fungus (e.g. Francis and Read, 1984; Grime *et al.*, 1987; Simard *et al.*, 1997). For ectomycorrhizae this may be a reliable method; the sheath of hyphes around the roots is an impermeable layer that isolates the roots from the surrounding soil in this type of mycorrhizae. (Smith and Smith, 1990). For arbuscular mycorrhizae the percentage of roots covered by the fungus does not provide a clear indication about the functionality of the association (Giovannetti en Sbrana, 1998). To be sure about the success of infection microscopic analysis of the roots is necessary.

The death of root cells following infection by an inappropriate fungus shows similarity with apoptosis (Alberts et al., 1994: 1076) as defense mechanism against phytopathogenic fungi where

root cells die and further infection is inhibited by the production of phytoalexines (Taiz and Zeiger, 1991: 336). I am not aware of research carried out on the production of these compounds in plants that are infected by arbuscular fungi, but if these compounds are being metabolized that may be an extra indication for the species specificity of the association between fungus and host plant.

### *Carbon allocation in the shoots of receiving plants*

The relative uptake of label in the shoots of the mycorrhizous plants in the experiment of Francis and Read (1984) was less when shadow was applied to the receiving plants. This is the opposite of what could be expected if the plants would fulfil their carbon requirements by uptake of carbon by the roots, according to Robinson and Fitter (1999). Transfer of sugars through the phloem occurs by means of bulk flow though and the differences found in label can be accounted for by transpiration, which could have been less in shadowed conditions, rather than in full light. At some stage in their growth, leaves change from sinks into carbon sources. The symplastic pathway for import of carbohydrates from the phloem is blocked physically and after that it is no longer possible to transfer sugars from the phloem into the leaf. If a mature leaf becomes a sink again, for example as a result of less illuminated conditions, the sugars remain in the phloem (Taiz and Zeiger, 1991: 164).

In the experiment by Francis en Read (1984) plants were exposed to label for two days, immediately followed by analysis of the shoots. Even if the plants were making new leaves, only minor amounts of label could have been assimilated in the leaves. This would be the result of the rate of carbon being transported into the shoot and the short period between the start of the labeling period and the measurements (Francis and Read, 1984; Farrar, 1985; Jones and Darrah, 1992).

Apart from the period of labeling and the time between labeling and measurement, the growth rate, and thus the season and the plant species used can be of influence on the amount and the rate at which carbon is being assimilated in the shoot. This has to be accounted for in future experiments.

## Conclusion

Proof of the existence of carbon transfer between plants via mycorrhizae is not provided in one experiment yet, but the results of several, separate experiments indicate that the criteria can be met. Transfer of carbon to the fungus occurs and it is clear that this carbon is transferred towards other plants. Uptake of carbon by receiving plants has been shown only on several occasions, the clearest in ectomycorrhizal associations. One explanation is that the plants grew only a little during the experiments. A possible extra explanation for the research conducted on arbuscular mycorrhizae is the very intimate cohabitation that can cause a self-defensive response of the plant if the combination of fungus and plant species does not match perfectly.

The experiment that unequivocally shows the existence of carbon transfer between plants interconnected by common mycorrhizal networks has to be carried out still. Until now the results of Grime *et al.* (1987), Simard *et al.* (1997) and Van der Heijden *et al.* (1998) are the strongest evidence available. Unequivocal proof requires an experimental design that combines several good properties of previous experiments, like the use of stable isotopes and inclusion of non-micorrhizous species.

## Literature


**Alberts, B., D. Bray, J. Lewis, M. Raff, K. Roberts and J. D. Watson, 1994.** Molecular Biology of the Cell, 3rd edition. Garland Publishing, New York.

**Aziz, T., D.M. Sylvia and R.F. Doren, 1995.** Activity and species composition of arbuscular mycorrhizal fungi following soil removal. Ecol. Applications 5(3): 776-784.

**Brownlee, C., J.A. Duddridge, A. Malibari and D.J. Read, 1983.** The structure and function of mycelial systems of ectomycorrhizal roots with special reference to their role in forming inter-plant connections and providing pathways for assimilate and water transport. Plant Soil 71: 433-443.

**Dickson, S. and P. Kolesik, 1999.** Visualisation of mycorrhizal fungal structures and quantification of their surface area and volume using laser scanning confocal microscopy. Mycorrhiza 9: 205-213.

**Duddridge, J.A., A. Malibari and D.J. Read, 1980.** Structure and function of mycorrhizal rhizomorphs with special reference to their role in water transport. Nature 287: 834-836.

**Ek, H., S. Andersson and B. Söderström, 1996.** Carbon and nitrogen flow in silver birch and Norway spruce connected by a common mycorrhizal mycelium. Mycorrhiza 6: 465-467.

**Farrar, J.F., 1985.** Fluxes of carbon in roots of barley plants. New Phytol. 99: 57-69.

**Fisher Walter, L.E., D.C. Hartnett, B.A.D. Hetrick and A.P. Schwab, 1996.** Interspecific nutrient transfer in a tallgrass prairie plant community. Am. J. Bot. 83(2): 180-184.

**Fitter, A.H., J.D. Graves, N.K. Watkins, D. Robinson and C. Scrimgeour, 1998.** Carbon transfer between plants and its control in networks of arbuscular mycorrhizas. Funct. Ecol. 12: 406-412.

**Francis, R., R.D. Finlay and D.J. Read, 1986.** Vesicular-arbuscular mycorrhiza in natural vegetation systems IV. Transfer of nutrients in inter- and intra-specific combinations of host plants. New Phytol. 102: 103-111.

**Francis, R. and D.J. Read, 1984.** Direct transfer of carbon between plants connected by vesicular-arbuscular mycorrhizal mycelium. Nature 307: 53-56.

**Gianinazzi-Pearson, V., S.E. Smith, S. Gianinazzi and F.A. Smith, 1991.** Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhizas - V Is $H^+$-ATPase a component of ATP-hydrolysing enzyme activities in plant-fungus interfaces? New Phytol. 177: 61-74.

**Giovannetti, M., C. Sbrana and C. Logi, 1994.** Early processes involved in host recognition by arbuscular mycorrhizal fungi. New Phytol. 127: 703-709.

**Giovannetti, M. and C. Sbrana, 1998.** Meeting a non-host: the behaviour of AM fungi. Mycorrhiza 8: 123-130.

**Graves, J.D., N.K. Watkins, A.H. Fitter, D. Robinson and C. Scrimgeour, 1997.** Intraspecific transfer of carbon between plants linked by a common mycorrhizal network. Plant Soil 192: 153-159.

**Grime, J.P., J.M.L. Mackey, S.H. Hillier and D.J. Read, 1987.** Floristic diversity in a model system using experimental microcosms. Nature 328: 420-422.

**Van der Heijden, M.G.A., J.N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken and I.R. Sanders, 1998.** Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396: 69-72.

**Jones, D.L. and P.R. Darrah, 1992.** Re-sorption of organic components by roots of *Zea mays* L. and its consequences in the rhizosphere I. Re-sorbtion of $^{14}C$ labelled glucose, mannose and citric acid. Plant Soil 143: 259-266.

**Jones, D.L. and P.R. Darrah, 1996.** Re-sorption of organic compounds by roots of *Zea mays* L. and its consequences in the rhizosphere. Plant Soil 178: 153-160.

**Jordy, M.N., S. Azémar-Lorentz, A. Brun, B. Botton and J.C. Pargney, 1998.** Cytolocalization of glycogen, starch, and other insoluble polysaccharides during ontogeny of *Paxillus involutus-Betula pendula* ectomycorrhizas. New Phytol. 140: 331-341.

**Marx, C., J. Dexheimer, V. Gianinazzi-Pearson and S. Gianinazzi, 1982.** Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhizas – IV Ultracoytoenzymological evidence (ATPase) for active transfer processes in the host-arbuscule interface. New Phytol. 90: 37-43.

**Morton, J.B. and S.P. Bentiventa, 1994.** Levels of diversity in endomycorrhizae (*Glomales, Zygomycetes*) and their role in defining taxonomic and non-taxonomic groups. Plant Soil 159: 47-59.

**Moora, M. and M. Zobel, 1998.** Can arbuscular mycorrhiza change the effect of root competition between conspecific plants of different ages? Can. J. Bot. 76: 613-619.

**Newman, E.I. and P. Reddell, 1987.** The distribution of mycorrhizas among families of vascular plants. New Phytol. 106: 745-751.

**Newman, E.I., 1988.** Mycorrhizal links between plants: their functioning and ecological significance. Adv. Ecol. Res. 18: 243-270.

**Peat, H.J. and Fitter, A.H., 1993.** The distribution of arbuscular mycorrhizas in the Brittish flora. New Phytol. 125: 845-854.

**Richardson, D.M., N. Allsopp, C.M. D'Antonio, S.J. Milton and M. Rejmanek, 2000.** Plant invasions - the role of mutualisms. Biol. Rev. 75: 65-93.

**Robinson, D. and A. Fitter, 1999.** The magnitude and control of control of carbon transfer between plants linked by a common mycorrhizal network. J. Exp. Bot. 50: 9-13.

**Simard, S.W., D.A. Perry, M.D. Jones, D.D. Myrold, D.M. Durall and R. Molina, 1997.** Net transfer of carbon between ectomycorrhizal tree species in the field. Nature 388: 579-582.

**Smith, F.A. and S.E. Smith, 1997.** Tansley Review No. 96: Structural diversity in (vesicular)-arbuscular mycorrhizal symbioses. New Phytol. 137: 373-388.

**Smith, S.E. and F.A. Smith, 1990.** Tansley Review No. 20: Structure and function of the interfaces in biotrophic symbioses as they relate to nutrient transport. New Phytol. 114: 1-38.

**Smith, S.E., V. Gianinazzi-Pearson, R. Koide and J.W.G. Cairney, 1994.** Nutrient transport in mycorrhizas: structure, physiology and consequences for efficiency of the symbiosis. Plant Soil 159: 103-113.

**Sun, Y.-P., T. Unestam, S.D. Lucas, K.J. Johanson, L. Kenne and R. Finlay, 1999.** Exudation-reabsorption in a mycorrhizal fungus, the dynamic interface for interaction with soil and soil microorganisms. Mycorrhiza 9: 137-144.

**Waters, J.R. and V.A. Borowicz, 1994.** Effect of clipping, benomyl and genet on $^{14}C$ transfer between mycorrhizal plants. Oikos 71: 246-252.

**Whittingham, J. and D.J. Read, 1982.** Vesicular-arbuscular mycorrhiza in natural vegetation systems III. Nutrient transfer between plants with mycorrhizal interconnections. New Phytol. 90: 277-284.

**Wilkinson, D.M., 1997.** The role of seed dispersal in the evolution of mycorrhizae. Oikos 78(2): 394-396.