

# Carbon partitioning in a split-root system of arbuscular mycorrhizal plants is fungal and plant species dependent

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## Summary

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- Root carbon (C) partitioning in two host plant species colonized by one of three arbuscular mycorrhizal (AM) fungal species was investigated.
- Split-root systems of barley (*Hordeum vulgare*) and sugar maple (*Acer saccharum*) were inoculated on one side with one of three AM fungi. Leaves were labelled with <sup>14</sup>CO<sub>2</sub> 3 wk after inoculation. Plants were harvested 24 h later and the root systems from the mycorrhizal (M) and nonmycorrhizal (NM) sides were analysed separately for <sup>14</sup>C.
- Partitioning of <sup>14</sup>C between M and NM sides varied depending on the fungal and host plant species used. *Gigaspora rosea* showed a strong C-sink capacity with both plant species, *Glomus intraradices* showed a strong C-sink capacity with barley, and *Glomus mosseae* did not affect <sup>14</sup>C partitioning. The C-sink strength of the M barley roots inoculated with *G. rosea* or *G. intraradices* was linearly correlated with the degree of colonization.
- The use of three AM fungal and two plant species allowed us to conclude that C-sink strength of AM fungi depends on both partners involved in the symbiosis.

**Key words:** arbuscular mycorrhizal (AM) fungi, carbon sink, colonization levels, barley (*Hordeum vulgare*), sugar maple (*Acer saccharum*), *Glomus intraradices*, *Glomus mosseae*, *Gigaspora rosea*.

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## Introduction

The roots of most terrestrial plants are symbiotically associated with obligate biotrophic fungi in the order Glomales (Zygomycotina) (Hayman, 1983). This arbuscular mycorrhizal (AM) association improves plant mineral nutrition (in particular phosphorus (P)), and can influence water uptake and resistance towards root pathogens (Smith & Read, 1997). In return, the plant supplies AM fungi with carbohydrates derived from photoassimilation (Ho & Trappe, 1973).

It has been demonstrated that photoassimilation and subsequent carbon (C) supply to the root system are closely linked to the development of AM fungi in the roots. Thomson *et al.* (1990) established a positive correlation between mycorrhizal (M) colonization levels and the concentration of soluble carbohydrates in subterranean clover roots colonized

by either *Scutellospora calospora* or *Glomus fasciculatum*. Moreover, Vierheilig *et al.* (2002) recently showed that bean plants inoculated with *Glomus mosseae* and grown in the dark did not become colonized. Wright *et al.* (1998b) demonstrated, using *Trifolium repens*, that M plants show higher photosynthetic rates than their nonmycorrhizal (NM) counterparts, but that this enhanced photoassimilation did not result in increased plant growth. Thus, the authors concluded that the C gain observed in M plants is probably channelled to the C sink developed by the mycobiont. Estimated C costs of the AM symbiosis are well documented in the literature (Pang & Paul, 1980; Kucey & Paul, 1982; Snellgrove *et al.*, 1982; Koch & Johnson, 1984; Douds *et al.*, 1988; Wang *et al.*, 1989) and many authors consider that 4–20% of the total C fixed by an AM plant is used by the mycobiont (Bago *et al.*, 2000; Douds *et al.*, 2000; Graham, 2000).

Considering the improved mineral acquisition efficiency of M plants compared with NM plants, the growth of M plants in general is improved. Phosphorus is a key element in the photosynthesis (Salisbury & Ross, 1985; Marschner, 1990) and therefore reduced P contents in the shoot (e.g. in NM plants) may directly affect plant growth. Furthermore, high foliar phosphate concentrations enable the translocation of C compounds towards other plant organs (Herold, 1980). Thus, changes in the nutritional status of M plants may result in a changed C budget. Because of the different nutritional status of M and NM plants it is therefore difficult to assess the cost of the M symbiosis by comparing M and NM plants (Pang & Paul, 1980). One possible solution to this problem is to supply P to NM plants as a means of obtaining plants of a similar size and P content (Kucey & Paul, 1982; Snellgrove *et al.*, 1982; Wright *et al.*, 1998a,b). However, as arbuscular mycorrhization not only affects P uptake but also the uptake of a wide range of nutrients such as nitrogen (N), sulphur (S), copper (Cu), zinc (Zn) or nickel (Ni) (Marschner, 1990; Smith & Read, 1997), results obtained using this experimental approach must also be interpreted with caution. Moreover, M plants have been shown to be more resistant towards soil-borne pathogens (Dehne, 1982; St-Arnaud *et al.*, 1995), indicating metabolic (not necessarily nutrient induced) changes. In order to overcome the differences between M and NM plants, a number of studies have used split-root systems (Koch & Johnson, 1984; Douds *et al.*, 1988; Wang *et al.*, 1989). Using this technique, the plant's root system is equally divided between two compartments, one of which is subsequently inoculated with a M fungus, hence allowing a comparison of the C-sink strength of M and NM roots of the same plant.

Most studies on C partitioning in M plants have not emphasized the possible importance of the effect of the fungal species and the host plant involved in the AM symbiosis. However, Pearson *et al.* (1993, 1994) showed that *S. calospora* and *Glomus* sp. (WUM 10(1)) exhibit different colonization patterns in relation to root carbohydrate concentration. More recently, van der Heijden *et al.* (1998), in a study of the impact of fungal diversity on plant diversity, showed that different plant species benefit from different AM fungal species. In an attempt to investigate some of the possible mechanisms underlying these findings, we studied the  $^{14}\text{C}$  partitioning in M and NM split-root systems of two economically important plants, barley and sugar maple, to estimate the C-sink strength capacity of each of three AM fungi.

## Materials and Methods

### Experimental design

The split-root system developed by Wyss *et al.* (1991) and modified by Vierheilig *et al.* (2000) was used. Two compartments, compartments B and C, contained the two halves of the root system of the study plant. An inoculum

compartment, compartment A, which comprised bean plants (*Phaseolus vulgaris* L. cv. Sun Gold) inoculated with one of the AM fungi under study (see below) or not (control) was attached to compartment B, which contained the half of the root system to be inoculated. A Nylon screen (60  $\mu\text{m}$  mesh) separated compartments A and B and compartments B and C were separated by a polyvinylchloride (PVC) plate.

### Biological material and growing conditions

The AM fungi *Gigaspora rosea* Nicolson & Schenck (DAOM 194757, ECORC, Agriculture and Agri-Food Canada, Ottawa, Canada), *Glomus intraradices* Smith & Schenck (DAOM 197198) and *G. mosseae* (Nicolson & Gerdemann) Gerd. & Trappe (BEG 12; La Banque Européenne des Glomales, International Institute of Biotechnology, Kent, UK) were used.

Barley seeds (*Hordeum vulgare* L. cv. Salome) were germinated in vermiculite. After 3 d, seedlings were transferred to the split-root system (two primary roots per compartment). Compartments B and C contained a steam-sterilized mixture of sand–soil–surface (1 : 1 : 1, vol : vol : vol). Plants were grown for 3 wk, in the presence of the inoculum compartment A, in a growth chamber (photoperiod 16 h; light 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR (photosynthetically active radiation); temperature 23°C/19°C day/night; relative humidity (r.h.) 50%) before labelling with  $^{14}\text{C}$ . To facilitate the obtaining of optimal M levels, no mineral fertilization was added.

Sugar maple seeds (*Acer saccharum* Marsh.) were collected in October 2000 in a sugar maple forest near Québec City. After 3 months of cold stratification (4°C), seeds were germinated in Perlite. Seedlings were transferred to pots (100 ml) containing a steam-sterilized mixture of sand–sugar maple forest soil–surface (1 : 2 : 1, vol : vol : vol) and grown under glass (photoperiod 14 h; light  $\geq 300 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR; temperature 25°C/17°C day/night; r.h. not controlled; no mineral fertilization). Six wk after germination, the primary root was cut 2–3 cm below the root–shoot interface to promote lateral root production. The seedlings were transferred to the split-root system 3 wk later. Plants were grown for 3 wk, in the presence of the inoculum compartment A, under the glass-house conditions outlined above, before labelling with  $^{14}\text{C}$ .

In order to increase the number of replicates, the experiment was repeated over time. Therefore, for both plant species, three repetitions of the experiment were performed with five replicates per treatment and per repetition giving a total of 15 plants for each of the four treatments (three fungal species and one control).

### $^{14}\text{CO}_2$ labelling

Compartment A was removed before labelling. Each plant shoot was placed inside a 945-ml (18 × 20 cm) transparent freezer bag (Ziploc, SC Johnson Inc., Racine, WI, USA) together with a 29.5-ml cup containing a basic solution of

37 kBq (1  $\mu$ Ci)  $\text{NaH}^{14}\text{CO}_3$  (Amersham Pharmacia Biotech, Cambridge, UK). The plastic bag was then closed and a sealing compound placed around the shoot stem. Gaseous  $^{14}\text{CO}_2$  was produced by injecting 1 ml lactic acid (85%) into the cup. Plants were exposed for 2 h in a growth chamber (see above for conditions). After the pulse period, the bags were removed under a venting fume-hood and the plants returned to a growth chamber.

After a 24-h chase period the two halves of the split-root systems were harvested separately and fresh weights recorded. For each repetition, subsamples of roots from two of the five replicates (plants) were collected from both compartments, weighed, and assessed for M colonization. The rest of the root systems were oven dried (24 h at 65°C), weighed, and used to determine the level of radioactivity. Barley roots were digested with the tissue solubilizer NCS (Amersham Pharmacia Biotech), and sugar maple roots were ground in liquid nitrogen and digested according to the technique described by Clifford *et al.* (1973). Radioactivity was assessed by liquid scintillation spectrometry. Counts were standardized with a quench curve and expressed in dpm. The presence of radioactivity in the substrate was determined from 1 g of the sand–soil–surface mix after digestion in NCS.

Results were expressed as a percentage of total  $^{14}\text{C}$  and as a percentage of total root dry weight in the M and NM compartments. Corrections were made for the root samples taken to determine M colonization levels.

### Effect of AM fungal species on growth of barley

The effect of *G. rosea*, *G. intraradices* and *G. mosseae* on the growth of barley was tested. Their effect on sugar maple was not tested in this study because this species showed a large, within-treatment growth variation in the previous split-root experiment.

The 4-d-old barley seedlings were transferred to compartment B (undivided) of the system described above. Four compartments, each containing 13 seedlings, were used. The seedlings of each compartment were inoculated, or not, by attaching inoculum compartments containing either M bean plants colonized by *G. rosea*, *G. intraradices* or *G. mosseae*, or NM plants, and grown in a growth chamber (see earlier for conditions). After 7 d, 10 plants of each of the four treatments were transferred to individual pots (700 ml) containing the steam-sterilized barley substrate (see above). The presence of M colonization was verified on the three remaining plants.

The pots were transferred to the greenhouse (see above for conditions). Plants were grown in a randomized complete-block design. Plants were watered weekly with a 10% Hoagland solution (200 ml per pot) and with deionized water as needed. In order to study the effect of the fungal treatments with time, harvests were performed after 4 wk and 8 wk. The M colonization levels (% root length colonized by M fungus) were determined for all plants on a fresh root sample. Shoots

were dried (24 h at 65°C) and weighed. Shoot dry weight was expressed as a percentage of the control.

### Measurement of mycorrhizal colonization levels

Barley root samples were stained using the ink and vinegar technique (Vierheilig *et al.*, 1998) and M colonization levels were measured according to Newman (1966). Sugar maple root samples were stained with Trypan blue (Koske & Gemma, 1989) and M colonization levels were assessed according to McGonigle *et al.* (1990). The M colonization levels were expressed as the percentage root length colonized by AM fungi regardless of the fungal structures.

### Statistical analyses

Split-root experiment data from barley and sugar maple were analysed separately. The data for the percentage of radioactivity allocated to each compartment were arcsin-transformed. Data from compartments B and C in the control plants were compared using a paired *t*-test to test the validity of our experimental design. The calculated *P*-values were 0.77 for barley and 0.72 for sugar maple, and showed no differences due to the experimental design. Therefore, further statistical analyses only considered the data from inoculated sides, which were analysed by two-way ANOVA, with treatment and repetition as main factors.

Shoot dry weight was analysed separately for the two harvests by one-way ANOVA, with fungal species as the treatment factor. *A posteriori* comparisons were made using LSD tests.

## Results

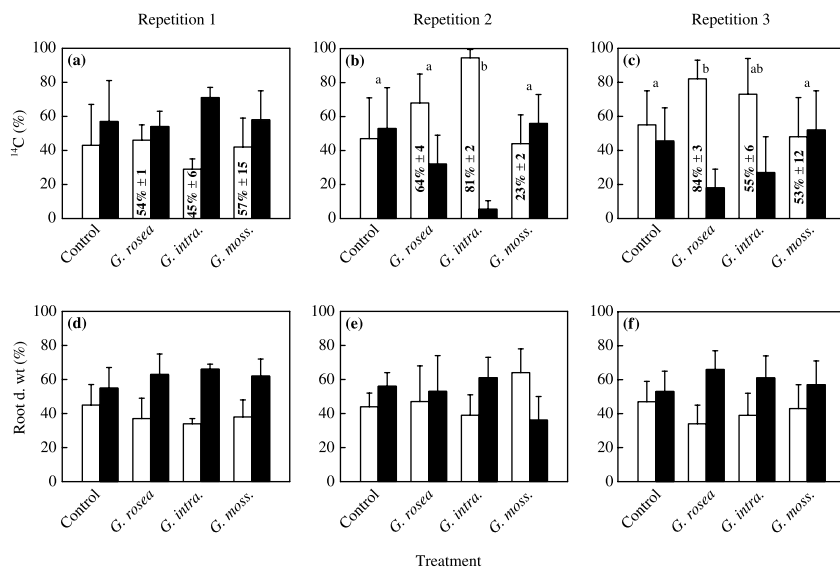
### Mycorrhizal colonization levels

The inoculated sides of the split-root systems of barley (Fig. 1a–c) and sugar maple (Fig. 2a) were colonized 3 wk after inoculation, regardless of the M fungus used. No colonization was observed in roots from NM control plants or in roots from the uninoculated compartment C of the split-root system.

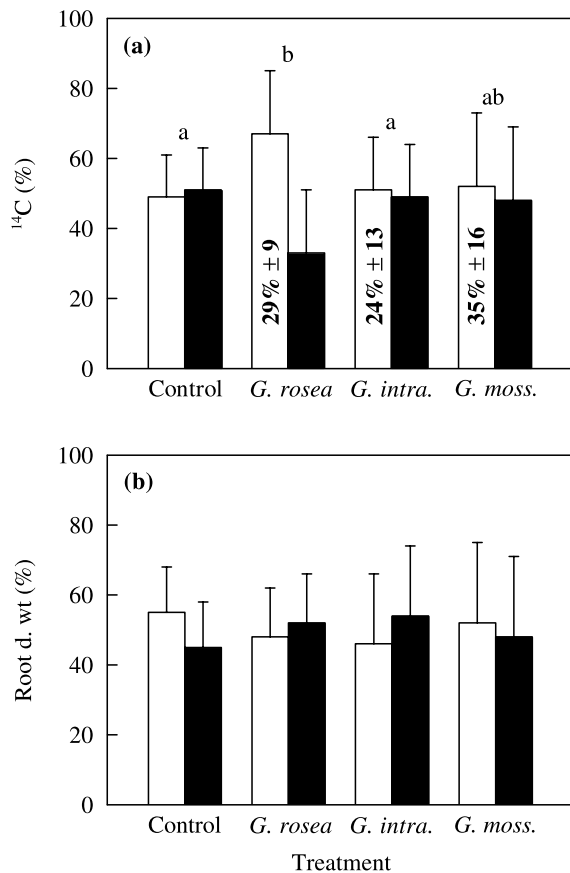
### $^{14}\text{C}$ partitioning

No radioactivity was detected in the growth substrate (< 60 dpm  $\text{g}^{-1}$  dry soil), therefore all the data analyses refer to the radioactivity measured in the roots.

**Barley host plants** The root systems of two barley plants were damaged during labelling. Consequently, the statistical analyses were performed on a total of 58 plants. The analysis of variance revealed strong treatment and repetition effects on  $^{14}\text{C}$  partitioning and a strong treatment  $\times$  repetition interaction (Table 1). Therefore, data obtained using the three



**Fig. 1** (a–c) Mean total  $^{14}\text{C}$  ( $\pm 1$  SD) partitioned and (d–f) mean percentage dry weight ( $\pm 1$  SD) of mycorrhizal (M) (open bars) and nonmycorrhizal (NM) (solid bars) barley roots, for each repetition, in control (compartments B and C), *Gigaspora rosea* (*G. rosea*), *Glomus intraradices* (*G. intra.*) and *Glomus mosseae* (*G. moss.*) treatments. Mycorrhizal colonization levels ( $\pm 1$  SD) are shown within M bars (a–c). When the ANOVA within the repetition was significant (b,c) an LSD test was performed (treatments with the same letter are not statistically different).



**Fig. 2** (a) Mean total  $^{14}\text{C}$  ( $\pm 1$  SD) partitioned and (b) mean percentage dry weight ( $\pm 1$  SD) of mycorrhizal (M) (open bars) and nonmycorrhizal (NM) (solid bars) sugar maple (*Acer saccharum*) roots in control (compartments B and C), *Gigaspora rosea* (*G. rosea*), *Glomus intraradices* (*G. intra.*) and *Glomus mosseae* (*G. moss.*) treatments. Mycorrhizal colonization levels ( $\pm 1$  SD) are shown within M bars (a). As treatment showed significant differences (a) an LSD test was performed (treatments with the same letter are not statistically different).

**Table 1** Degrees of freedom (df), mean sum of squares (MS), *F*- and *P*-values of (a) a two-way ANOVA and (b) one-way ANOVAs for each repetition performed on percentage  $^{14}\text{C}$  allocated to mycorrhizal barley (*Hordeum vulgare*) roots (data arcsin-transformed)

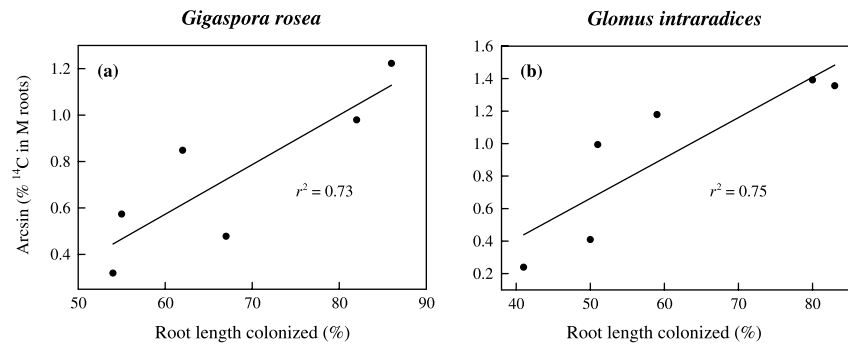
Source of variation	df	MS	<i>F</i>	<i>P</i>
(a) Treatment (Treat.)	3	0.3959	8.08	0.0002
Repetition (Repet.)	2	0.6994	14.27	0.0000
Treat. $\times$ repet.	6	0.2797	5.71	0.0002
Error	46	0.0490	–	–
(b) Repet. 1	Treat. 3	0.0349	1.04	0.4029
Repet. 2	Treat. 3	0.6567	13.74	0.0001
Repet. 3	Treat. 3	0.2337	3.61	0.0365

Error of df were 15 in repetitions 1 and 2, 16 in 3.

different fungi were analysed separately for each repetition. Significant one-way ANOVA tests were followed by an LSD test. The percentage of  $^{14}\text{C}$  allocated to M roots was significantly higher than control roots ( $P$ -value  $< 0.05$ ) in plants inoculated with *G. intraradices* in repetition 2 and with *G. rosea* in repetition 3 (Table 1, Fig. 1b,c). When inoculated with *G. mosseae*, the percentage of  $^{14}\text{C}$  allocated to M roots never differed significantly from control roots (Fig. 1a–c).

Partitioning of  $^{14}\text{C}$  in plants colonized by *G. rosea* and *G. intraradices* appeared to be correlated with M colonization levels: the higher the M colonization level, the stronger the sink for carbohydrates (Fig. 1a–c). By contrast,  $^{14}\text{C}$  partitioning in plants colonized by *G. mosseae* (Fig. 1a–c) was not affected by M colonization levels and was always equally divided between M and NM roots. Data from the six plants (two per repetition) used to determine M colonization levels were used to perform linear correlation plots representing arcsin ( $\%^{14}\text{C}$  in M roots/100) as a function of M colonization levels. The  $r^2$  values were 0.73 ( $P = 0.03$ ) for *G. rosea* (Fig. 3a) and 0.75

**Fig. 3** Relationship between percentage  $^{14}\text{C}$  allocated to mycorrhizal (M) roots (arcsin-transformed) and the percentage of barley root length colonized by (a) *Gigaspora rosea* and (b) *Glomus intraradices*. Dots represent the two out of five plants per repetition used to assess M colonization levels;  $r^2$  values of the correlation are presented.



( $P = 0.03$ ) for *G. intraradices* (Fig. 3b), confirming the presence of a significant correlation between these two parameters. By contrast, the  $r^2$  value was 0.37 ( $P = 0.20$ ) for *G. mosseae* (data not shown).

Increased  $^{14}\text{C}$  partitioning in M roots did not correspond to increased root dry weight when compared with NM roots (Fig. 1d–f). In fact, M roots often represented a lower percentage of the total root dry weight than their NM counterparts in all four treatments, but treatment effect on percentage root dry weight was not statistically significant ( $F = 2.65$ ,  $P = 0.06$ ).

**Sugar maple host plants** During the 3 wk following the inoculation, leaves of 23 of the sugar maple seedlings exhibited extensive necrotic zones or showed no capacity to fix  $^{14}\text{CO}_2$  (absence of  $^{14}\text{C}$  in the leaves after labelling). Consequently, the statistical analyses were performed on a total of 37 plants. The analysis of variance revealed a treatment effect on  $^{14}\text{C}$  partitioning but no effect of repetition and no treatment  $\times$  repetition interaction (Table 2). Therefore, data from all repetitions for each fungal species were pooled for comparison with the control data. *Gigaspora rosea* increased  $^{14}\text{C}$  partitioning towards M roots (Table 2, Fig. 2a) while root dry weight was not affected by mycorrhization (Fig. 2b). Furthermore, there was no correlation between percentage  $^{14}\text{C}$  in M roots and M colonization levels ( $r^2 = 0.01$ ,  $P = 0.88$ ). *Glomus intraradices* and *G. mosseae* neither modified  $^{14}\text{C}$  partitioning nor root dry weight between M and NM roots (Fig. 2).

**Table 2** Degrees of freedom (df), mean sum of squares (MS),  $F$ - and  $P$ -values of a two-way ANOVA performed on percentage  $^{14}\text{C}$  allocated to mycorrhizal sugar maple (*Acer saccharum*) roots (data arcsin-transformed)

Source of variation	df	MS	$F$	$P$
Treatment (Treat.)	3	0.1935	3.55	0.0288
Repetition (Repet.)	2	0.0061	0.11	0.8942
Treat. $\times$ repet.	6	0.0459	0.84	0.5501
Error	25	0.0545	–	–

### Growth of mycorrhizal barley

Significant differences in shoot dry weight were observed ( $P = 0.03$ ) 4 wk after transfer to individual pots. Shoot dry weight in *G. rosea* and *G. intraradices* treatments were significantly lower ( $63 \pm 14\%$  and  $66 \pm 12\%$ , respectively) than the control, while shoot dry weight of plants inoculated with *G. mosseae* did not differ from the other treatments ( $78 \pm 12\%$  of control). At the second harvest (8 wk after transfer to pots), significant differences in shoot dry weight were also observed ( $P = 0.02$ ). Shoot dry weight in the *G. intraradices* treatment was significantly lower ( $67 \pm 21\%$ ) than the control, while *G. rosea* and *G. mosseae* treatments did not differ from the other treatments ( $85 \pm 8\%$  and  $82 \pm 7\%$  of control, respectively). All the inoculated plants were extensively colonized by AM fungi (data not shown).

### Discussion

In the present study we have shown that fungal and plant species are major factors influencing C partitioning between M and NM roots grown in a split-root system. In previous experiments with similar split-root systems the effect of one AM fungal strain on the C partitioning between M and NM roots of one (Douds *et al.*, 1988; Wang *et al.*, 1989) or two plant species (Koch & Johnson, 1984) was studied. In our experiments, we used two morphologically and phenologically different host plant species: barley, a rapidly growing herbaceous annual and sugar maple, a slow-growing woody perennial. The ability of three different AM fungal species, from two genera, to alter the C-sink strength of the root system was tested on each of the two plant species. Thus, in our experiment, C partitioning in split-root systems was studied in six AM fungus-host plant combinations.

The C partitioning in M and NM roots of the two plant species showed different patterns depending on the AM fungal partner. The three AM fungi which were used not only showed different C-sink strength capacities but the sink strength of one, *G. intraradices*, also seemed to be host-plant dependent. The results for *G. intraradices* differ from those obtained by Koch & Johnson (1984), who showed that *G.*

*intraradices* had no effect on  $^{14}\text{C}$ -labelled photosynthate partitioning between M and NM roots in two citrus cultivars. In the present study, *G. rosea* and *G. intraradices* showed a strong C-sink capacity in barley. In both fungi the sink strength was positively correlated with colonization levels. Low root colonization resulted in no increase in C transfer to M roots. This is in accord with the results of Thomson *et al.* (1990), that showed a positive relationship between the soluble carbohydrate concentration in the roots of *Trifolium subterraneum* and the percentage of root colonization by *S. calospora* and *G. fasciculatum*. In our study, the variability of M levels between repetitions in barley was probably due to variations in the inoculation capacity of the M bean plants used (Pearson *et al.*, 1994). Compared with colonization levels in barley, root colonization by *G. rosea* in sugar maple seedlings was low. Nevertheless, the C transfer to M roots indicated that *G. rosea* was a strong C sink. However, unlike the experiment with barley, *G. intraradices*, with a similar level of root colonization to *G. rosea*, was not a strong sink for C in sugar maple and *G. mosseae* showed a low C-sink capacity in barley and sugar maple. The M levels in barley colonized by *G. mosseae* were low compared with *G. rosea* and *G. intraradices*, and this could explain the weak sink strength of this fungus. These M levels are, however, comparable with those reported in the study of Vierheilig *et al.* (2000) in which the same fungal strains and the same host plant (barley cv. Salome) were used. The present results thus support the idea that a given AM fungus may have totally different effects depending on the plant species (van der Heijden *et al.*, 1998) with which it is associated.

Differences in C partitioning in M and NM roots cannot be attributed to differences in root dry weight. As expected and as previously observed in fresh barley roots (Vierheilig *et al.*, 2000), the M root dry weight was lower than the NM root dry weight of barley plants colonized by *G. rosea* and *G. intraradices*. By contrast, in repetition 2, barley plants inoculated with *G. mosseae* showed higher M root dry weight than NM root dry weight. In sugar maple, mycorrhization had no impact on root dry weight, regardless of the AM fungus. This could perhaps be explained by the fact that sugar maple is a slow-growing tree and that the time between the mycorrhization and harvest of sugar maples might have been too short to detect differences in dry weight between M and NM compartments.

What is the implication of AM fungi of different sink strength on host plants? A permanent strong C-sink is probably unfavourable to plants in which growth is C limited (e.g. under low light conditions), but it is likely that the strength of the C sink decreases once the fungus is well established in the host roots and once it has extensively colonized the mycorrhizosphere. Conversely, a fungus with low C-sink strength is expected to be profitable to its host only if it efficiently improves the uptake of mineral nutrients. The study of the effects of the three AM fungi tested on the growth

and mineral nutrition of barley are complementary to the C partitioning experiments. It showed that the plants harvested at 4 wk had a decreased shoot dry weight when inoculated with the two species showing a strong C-sink capacity – *G. rosea* and *G. intraradices* – while *G. mosseae* did not cause any growth depression. After 8 wk, *G. intraradices* was the only AM fungus still suppressing plant growth. Analyses of mineral foliar concentrations did not give significant differences (data not shown). It appears that the mycorrhization of barley (cv. Salome) by the strong C sink *G. rosea* is costly during initial establishment and that it can deprive plants of notable amounts of C. This is probably linked with the fact that species within the genus *Gigaspora* rapidly form a dense and extensive mycelial network (Dodd *et al.*, 2000; Hart & Reader, 2002). Moreover, it has been noted that *S. calospora*, another member of the Gigasporaceae, has particularly high C requirement compared with *Glomus* species (Thomson *et al.*, 1990; Pearson *et al.*, 1993). *Glomus intraradices*, the other fungus showing a strong C-sink capacity in barley, probably used plant-derived C for the formation of large quantities of intraradical vesicles inside colonized roots (Peng *et al.*, 1993). Similar growth depression in M plants has previously been reported for *G. intraradices* (Peng *et al.*, 1993; Marschner & Crowley, 1996; Pozo *et al.*, 2002) and for many other AM fungal species (Schenck & Smith, 1982; Boyetchko & Tewari, 1995; Graham & Abbott, 2000; Taylor & Harrier, 2000). *Glomus mosseae* which apparently was a low C sink, also produced plants of slightly smaller size than the control. The present results suggest that AM C cost is influenced by the development state of the fungal partner and perhaps also by the developmental state of the plant.

In conclusion, this is, to the best of our knowledge, the first report of C partitioning in M and NM split-root systems involving different host plant species colonized by different AM fungi. This work emphasizes the importance of considering both plant and fungal species when studying the C-sink strength capacity of AM fungi or the C cost induced by M colonization. This study also highlights the fact that an individual AM fungus might be either a strong or weak C sink depending on the plant host. Furthermore, in several AM fungal species M colonization levels appear to be an important factor determining fungal C-sink strength. Finally, an expensive AM fungus may not necessarily be a disadvantage to a plant host if there are long-term fitness gains.

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