

## Variable carbon-sink strength of different *Glomus mosseae* strains colonizing barley roots

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**Abstract:** Root carbon (C) partitioning was investigated in barley (*Hordeum vulgare* L.) colonized by one of three strains of the arbuscular mycorrhizal fungus (AMF) *Glomus mosseae* (Nicolson & Gerdemann) Gerd. & Trappe. The roots of each plant were evenly divided between two compartments of a split-root system and one side was inoculated with one of the three AMF strains. Twenty-three days after inoculation barley shoots were labeled with  $^{14}\text{CO}_2$ . Twenty-four hours later, plants were harvested and the mycorrhizal (M) and nonmycorrhizal (NM) roots were analyzed separately for  $^{14}\text{C}$ . Partitioning of C between M and NM sides differed between the fungal strains: BEG 54 was a strong C sink, BEG 55 was a moderately strong C sink, and BEG 12 showed similar C-sink strength as the non-inoculated control plants. The observed differences in C-sink strength mirrored differences in plant dry biomass. Total plant dry biomass of plants inoculated with BEG 12, BEG54, and BEG 55 represented 81.3%, 65.3%, and 73.4% of the biomass of the control plants, respectively. This paper is the first report of an AMF strain-specific variation of C partitioning in M plants in a split-root system.

**Key words:** arbuscular mycorrhizal fungi, barley (*Hordeum vulgare*), carbon sink, *Glomus mosseae*, strain variability.

**Résumé :** La répartition racinaire de carbone (C) a été étudiée chez de l'orge (*Hordeum vulgare* L.) colonisée par une parmi trois souches du champignon mycorrhizien à arbuscules (MA) *Glomus mosseae* (Nicolson & Gerdemann) Gerd. & Trappe. Les racines de chaque plante ont été équitablement réparties entre les deux compartiments d'un système en racines dédoublées et un côté a été inoculé avec l'une des trois souches du champignon MA. Vingt-trois jours après inoculation, les parties aériennes d'orge ont été marquées avec du  $^{14}\text{CO}_2$ . Vingt-quatre heures plus tard, les plantes ont été récoltées et le  $^{14}\text{C}$  a été analysé séparément dans les racines mycorrhizées (M) et non mycorrhizées (NM). La répartition du  $^{14}\text{C}$  entre les côtés M et NM variait entre les souches fongiques: BEG 54 était un puits de C fort, BEG 55 était un puits de C modéré alors que BEG 12 montrait une force de puits similaire à celle des plantes témoins. Les différences de force de puits de C observées se reflétaient en différences de biomasse végétale sèche. La biomasse végétale sèche totale des plantes inoculées par BEG 12, BEG 54 et BEG 55 représentait respectivement 81,3 %, 65,3 % et 73,4 % de la biomasse des plantes témoins. Cet article rend compte pour la première fois d'une variation de souche dans la répartition du C chez des plantes M dans un système en racines dédoublées.

**Mots clés :** champignons mycorrhiziens à arbuscules, orge (*Hordeum vulgare*), puits de carbone, *Glomus mosseae*, variabilité de souche.

### Introduction

Arbuscular mycorrhizal fungi (AMF) belong to a ubiquitous group of Zygomycetes and form a symbiotic association with the roots of most land plants. AMF improve plant

nutrition mainly by transferring phosphorus (P) from the soil to the plant. The host plants provide the fungi with carbohydrates (Smith and Read 1997).

The variability of the effect of different AMF genera, species, or even different strains of the same species on plant nutritional parameters, such as growth and P acquisition, is well documented (Graham et al. 1982; Giovannetti and Gianinazzi-Pearson 1994; Smith et al. 2000; Burleigh et al. 2002); however, few data are available concerning the variability of the carbohydrate demand of different AMF. Carbon (C)-sink strength studies by Pang and Paul (1980), Kucey and Paul (1982), Snellgrove et al. (1982), Koch and Johnson (1984), Douds et al. (1988) and Wang et al. (1989) suggest that the cost of supporting AMF symbionts represents 4% to 20% of the total carbon fixed by the plant. In these studies, experimental conditions or the AMF used varied and therefore no comparison of C-sink strength between AMF has been possible. However, Pearson and Jakobsen

Received 12 November 2002. Published on the NRC Research Press Web site at <http://canjbot.nrc.ca> on 30 August 2003.

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(1993) compared the overall consumption of  $^{14}\text{CO}_2$  in plants colonized by one of three species of AMF and found differences between treatments in the proportion of  $^{14}\text{C}$  allocation below ground.

The C-sink strength of three AMF, *Gigaspora rosea*, *Glomus intraradices*, and *Glomus mosseae*, was compared in a recent study using a split-root system (Lerat et al. 2003). The split-root system allows the study of C partitioning between mycorrhizal (M) and nonmycorrhizal (NM) roots of the same plant and thus avoids the comparison of M and NM plants with a potentially different mineral status. Using this system with barley and sugar maple, Lerat et al. (2003) observed clear differences in the sink strength of the tested fungi. *Gigaspora rosea* exhibited a high sink strength capacity in both host plants and *G. intraradices* had a strong sink capacity in barley but not in sugar maple. These results therefore showed that the sink strength of an AMF can vary between different host plants. The third AMF, *G. mosseae*, showed a low sink strength in both plant species tested. The C-sink strength of AMF can thus vary greatly between fungal species.

The goal of the present work was to investigate, using three strains of *G. mosseae* from different geographic regions, whether the low sink strength of *G. mosseae* colonized roots is a common feature of this species or whether it is strain specific.

## Materials and methods

### Biological material and growing conditions

Barley (*Hordeum vulgare* L. 'Salome') seeds were surface-sterilized in 0.75% sodium hypochlorite (5 min), rinsed with tap water, and germinated in vermiculite.

After 4 days the seedlings were transferred to a split-root system (two roots per compartment) containing a steam-sterilized (40 min, 120 °C) mixture of silicate sand, Turface® (Profile Products LLC, Buffalo Grove, Ill.), and agricultural soil (v:v:v:1:1:1). After transfer of the barley plants, roots on one side of the split-root system were inoculated with one of the three tested *G. mosseae* strains using the inoculum compartment method of Vierheilig et al. (2000a). Briefly, the inoculum compartments contained bean plants (*Phaseolus vulgaris* L. 'Sun Gold') colonized by one of three *Glomus mosseae* (Nicolson & Gerdemann) Gerd. & Trappe strains identified by their BEG (La Banque Européenne des Glomales; International Institute of Biotechnology; Kent; U.K.) number: (i) BEG 12; isolated in England, (ii) BEG 54, isolated in Indonesia, and (iii) BEG 55, isolated in the Philippines. The inoculum compartments and the split-root compartments are separated by a nylon screen that allows fungal hyphae, but not roots, to pass. Control plants were in contact with an inoculum compartment containing NM bean plants. The plants were grown in a growth chamber (photoperiod 16 h light: 8 h dark at a temperature of 23:19 °C (light:dark)) at 300  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  photosynthetically active radiation (PAR), relative humidity 50%, with no additional mineral fertilization.

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

Twenty three days after inoculation, the inoculum compartments were removed and the barley shoots were labeled

with 37 kBq (1  $\mu\text{Ci}$ )  $^{14}\text{CO}_2$  for 2 h as described in Lerat et al. (2003). After a 24-h chase period the two halves of each split-root system were harvested separately and the fresh biomass recorded. Mycorrhizal roots of each plant were subsampled, weighed, and used to assess mycorrhizal colonization levels. The rest of the root systems were oven dried, weighed, and used to determine radioactivity levels. Shoots were oven dried and weighed. Dried roots were digested with tissue solubilizer (NCS) at 60 °C overnight. 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 of inoculated and non-inoculated compartments was also assessed after digestion of approximately 1 g of substrate in NCS. The experiment was repeated twice with five replicates per repetition and per treatment giving a total of 10 plants per treatment.

Carbon partitioning results were expressed as percentage of total  $^{14}\text{C}$  and as percentage of total root dry biomass in the inoculated and non-inoculated compartments. Corrections were made for root subsamples used for the determination of M colonization levels. Because dry biomass differed between repetitions, shoot, root, and total dry biomass were expressed as a percentage of the mean of the control plants within repetition.

### Measurement of root colonization

Mycorrhizal root samples from each inoculated plant and randomly selected NM root samples were cleared by boiling in 10% KOH and stained by boiling in a 3% ink (Shaeffer; black) – vinegar (5% acetic acid) solution (Vierheilig et al. 1998; Vierheilig et al. 2000b). Stained roots were observed with a light microscope and the percentage of root colonization was determined using the method of Newman (1966).

### Statistical analyses

The percentages of radioactivity allocated to each of the M and NM compartments were arcsin transformed.  $^{14}\text{C}$  partitioning to the inoculated sides (M compartment) was compared using two-way ANOVA, with *G. mosseae* strain as the treatment factor and repetition in time (block) as the other factor. Shoot, root, and total dry biomass were analyzed by two-way ANOVA, with fungal strain as the treatment factor and repetition in time (block) as the other factor. A posteriori comparisons were made using LSD tests.

## Results

Three weeks after inoculation, the inoculated sides of the split-root systems of barley were heavily colonized regardless of the *G. mosseae* strain used (Fig. 1a). No colonization was observed in non-inoculated root samples.

No radioactivity was detected in the growth substrate (<60 dpm·g<sup>-1</sup> dry soil); therefore, all the data analyses refer to the radioactivity measured in the roots.

$^{14}\text{C}$  partitioning was affected by treatment ( $P = 0.01$ ) but not by repetition ( $P = 0.66$ ). Greater amounts of  $^{14}\text{C}$  were found in M roots of the BEG 54 treatment compared with the control and BEG 12 treatments, while the partitioning of  $^{14}\text{C}$  towards M roots of the BEG 55 treatment was not significantly different from partitioning observed in the other

**Table 1.** Dry biomass ( $\pm 1$  SD) of control and *Glomus mosseae* BEG 12, BEG 54, and BEG 55 treatments expressed as percentage of control.

Dry biomass	Control	BEG 12	BEG 54	BEG 55
Shoot*	100.0 $\pm$ 24.3a	74.6 $\pm$ 11.3b	59.6 $\pm$ 12.0b	72.7 $\pm$ 14.4b
Roots <sup>†</sup>	100.0 $\pm$ 12.9a	95.7 $\pm$ 21.1a	80.8 $\pm$ 31.3b	77.5 $\pm$ 23.7b
Total <sup>‡</sup>	100.0 $\pm$ 19.8a	81.3 $\pm$ 9.1b	65.3 $\pm$ 15.4c	73.4 $\pm$ 14.8bc

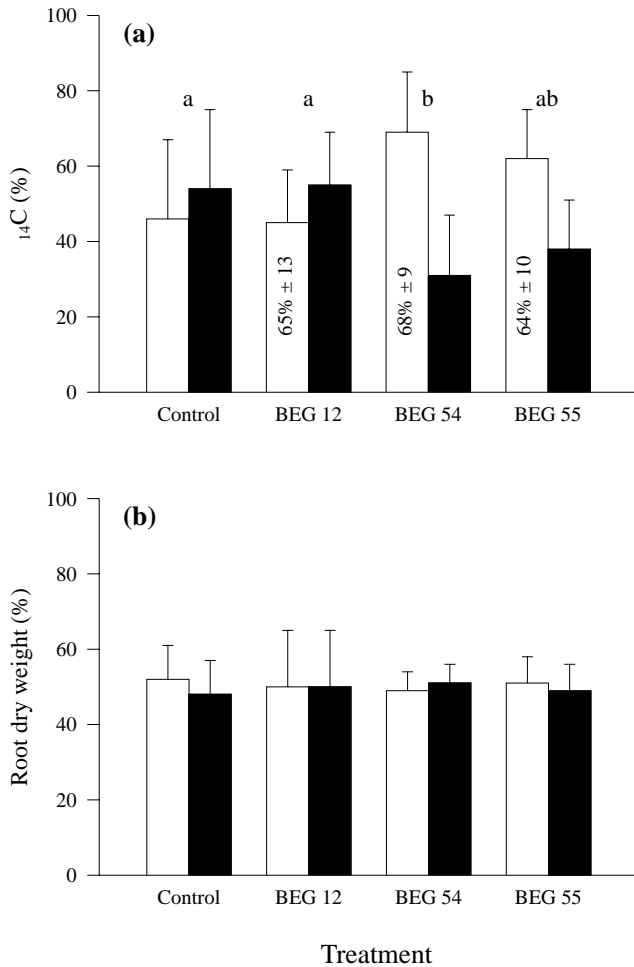
**Note:** Means are calculated from the sum of the two repetitions. In repetition 1, shoot and root biomass of control plants were 390  $\pm$  70 mg and 238  $\pm$  20 mg, respectively, while in repetition 2, shoot and root biomass of control plants were 701  $\pm$  273 mg and 262  $\pm$  49 mg, respectively. Treatments within a row with the same letter are not statistically different.

\*Results of the two-way ANOVA: treatment,  $P = 0.00$ ; repetition,  $P = 0.70$  and interaction (treatment $\times$ repetition),  $P = 0.32$ .

<sup>†</sup>Results of the two-way ANOVA: treatment,  $P = 0.01$ ; repetition,  $P = 0.00$  and interaction,  $P = 0.00$ .

<sup>‡</sup>Results of the two-way ANOVA: treatment,  $P = 0.00$ ; repetition,  $P = 0.07$  and interaction,  $P = 0.02$ .

**Fig. 1.** (a) Mean total  $^{14}\text{C}$  ( $\pm 1$  SD) partitioning and (b) mean percentage dry biomass ( $\pm 1$  SD) between M (open bars) and NM (solid bars) barley roots in control and *Glomus mosseae* BEG 12, BEG 54, and BEG 55 treatments. Means are calculated from the sum of the two repetitions. Treatments with the same letter are not statistically different. Mycorrhizal colonization levels ( $\pm 1$  SD) are shown within M bars (a).



treatments (Fig. 1a). A paired  $t$ -test analysis (results not shown) revealed no significant differences between root dry biomass of M and NM sides (data arcsin-transformed) in any treatment (Fig. 1b).

In all fungal treatments, total and shoot dry biomass were lower than the control (Table 1). The BEG 54 treatment generated significantly smaller plants than the BEG 12 treatment, while the BEG 55 plants did not significantly differ in size from the two other fungal treatments. Root dry biomass was unaffected by the BEG 12 treatment; however, the BEG 54 and BEG 55 treatments produced less root dry biomass than the control plants.

## Discussion

While the existence of species-specific differences in the C-sink strength capacity of AMF has been shown (Pearson and Jakobsen 1993; Lerat et al. 2003), the present study using three *G. mosseae* strains confirms that differences in C-sink strength may also occur at the intraspecific level. The *G. mosseae* strain BEG 12 showed a low C-sink strength capacity, which confirms the results obtained by Lerat et al. (2003). The other two *G. mosseae* strains, BEG 54 and BEG 55, showed C-sink strength capacities similar to those described for two other AMF species, *G. intraradices* and *Gigaspora rosea* (Lerat et al. 2003). The differences observed in the C-sink strength capacity between the *G. mosseae* strains were correlated to differences in plant biomass. BEG 12, the strain with the lowest sink strength capacity, depressed barley total plant biomass the least, while BEG 54, the strain with the highest sink strength capacity, induced the largest plant biomass suppression. Previous studies have also demonstrated that plants colonized by certain AMF may show no growth stimulation and may even show a reduction in growth when compared with non-inoculated controls (e.g., Marschner and Crowley 1996; Pozo et al. 2002; Lerat et al. 2003). This may be linked to the aggressiveness of the fungus with regard to its ability to colonize the root system and to the level of its requirement for host plant-derived C (Graham and Abbott 2000; Pozo et al. 2002). As AMF are obligate biotrophs depending on host plant C for establishment of the symbiosis and subsequent growth and nutrient uptake (Smith and Read 1997; Vierheilig et al. 2002), the measurements of C-sink strength capacity of a given mycobiont has the potential to provide an insight into the metabolic activity (including respiration) and the extraradical hyphal development and spore production of the fungus. Different C-sink strength may explain intraspecific AMF growth differences (Graham et al. 1982; Giovannetti and Gianinazzi-Pearson 1994).

The different C-sink strength capacities observed are likely to reflect strain specific genetic variations. Although Lanfranco et al. (1995) and Lloyd-Macgilp et al. (1996) showed intra-specific genetic variability for a number of AMF, the analysis of ribosomal DNA of various strains of *G. mosseae* has revealed this species to be genetically similar world-wide (Lloyd-Macgilp et al. 1996). This suggests that this technique is insufficient to reveal species-specific differences in metabolic capacity. However, recently developed DNA-microarray techniques will allow the mechanisms underlying the C-sink strength capacities of different AMF to be studied in greater depth.

In conclusion, the present study has shown that the weak C-sink strength of *G. mosseae* BEG 12 reported by Lerat et al. (2003) is strain- and not species-specific.

## Acknowledgements

The authors wish to thank Dr. John C. Dodd (Kent, U.K.) for kindly providing BEG 54 and BEG 55 strains and Andrew P. Coughlan for the revision of this text. This work was supported by the Centre de Recherche en Biologie Forestière (CRBF) and by Natural Sciences and Engineering Research Council of Canada (NSERC) grants to L.L. and Y.P.

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