

## Short Communication

# Further root colonization by arbuscular mycorrhizal fungi in already mycorrhizal plants is suppressed after a critical level of root colonization

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Received March 10, 2003 · Accepted June 17, 2003

## Summary

An established arbuscular mycorrhizal symbiosis suppresses further mycorrhization. It is not clear whether the observed suppressional effect is linked with the level of root colonization or not. In the present work we studied the effect of the degree of root colonization by the arbuscular mycorrhizal fungus *Glomus mosseae* on further root colonization by *G. mosseae*.

At different time points barley plants grown in split-root compartments were pre-inoculated on one half of the split-root system with *G. mosseae*. Sequential inoculation resulted in different colonization levels. Thereafter, the second half of the split root system was inoculated. The results indicate an enhanced suppression of root colonization on the second side of the split-root system when colonization levels increased on the first side.

**Key words:** Arbuscular mycorrhiza – autoregulation – barley – glomales

## Introduction

The arbuscular mycorrhizal symbiosis is an association between plant roots and fungi in more than 80 % of all land plants. Legumes establish symbiosis not only with arbuscular mycorrhizal fungi (AMF) but also with nodule-forming rhizobia. In legumes, after a critical number of nodules are formed, suppression of further nodulation occurs, a phenomenon termed autoregulation (for details see review in Caetano-Anollés and Gresshoff 1991). Similarly, in mycorrhizal plants,

once an AMF has colonized roots, further root colonization by an AMF is reduced (Vierheilig et al. 2000 a, b), showing the presence of an autoregulatory mechanism also in the arbuscular mycorrhizal symbiosis.

Recently, it could be shown that an established mycorrhizal symbiosis not only suppresses further mycorrhization but also nodulation (Catford et al. 2003). In all experiments on AMF autoregulation (Vierheilig et al. 2000 a, b, Catford et al. 2003) one side of a split-root system was pre-inoculated with an AMF until the roots were heavily colonized by the fungus, and thereafter the second side of the split-root system was inoculated. Thus, it is not clear whether the observed suppress-

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sional effect is linked with the level of root colonization or not. In the present work we studied the effect of the degree of root colonization by *Glomus mosseae* on further root colonization by *G. mosseae*.

## Materials and Methods

### Biological material and growth conditions

Barley (*Hordeum vulgare* L. cv. Salome) seeds were surface-sterilized by soaking in 0.75 % sodium hypochlorite for 5 min, rinsed with tap water, and germinated in vermiculite. After three days, the seedlings were transferred to a steam-sterilized (40 min, 120 °C) mixture of silicate sand, TurFace® (baked clay substrate, which is mechanically broken to a diameter of approximately 2–5 mm; Applied Industrial Materials, Corp.; Buffalo Grove, Illinois, USA) and soil (vol : vol : vol/ 2 : 2 : 1) into a split-root system, as described previously (Vierheilig et al. 2000 a). Plants were watered every second day. Briefly, the split-root system consists of two units, each containing half of the barley root system. The two compartments are separated on the side joining each other by an impermeable PVC membrane in order to prevent any flow of molecules or root or hyphal growth from one side to the other. Thus one side of the split-root system can be inoculated with AMF without inoculating the other side. Experiments were performed in a growth chamber (day/night cycle: 16 h; 22 °C/8 h; 20 °C; rel. humidity 50%; light intensity 310  $\mu\text{E m}^{-2}\text{s}^{-1}$ ).

### Inoculation with AMF

The outer side of each split-root compartment is equipped with a nylon screen (60  $\mu\text{m}$  mesh), which can be penetrated by hyphae but not by roots. To inoculate half the the split barley root system with *G. mosseae*, the outer side of a split-root compartment was joined to an inoculum compartment also equipped with a nylon screen, thus hyphae from the inoculum compartment could colonize roots on one side of the split-root compartment.

The inoculum compartment contained beans (*Phaseolus vulgaris* L. cv. Sun Gold) colonized by *G. mosseae* (Nicolson & Gerdemann) Gerd. & Trappe (BEG 12; European Bank for the Glomales).

### Experimental design

Two days after transferring plants into the split-root compartments in the first treatment, five plants were immediately inoculated on side A of the split-root system with the AMF (time of inoculation before inoculation of second half B was 13 d). In the second treatment 9 d after transferring plants into the split-root compartments another 5 plants were inoculated (time of inoculation before inoculation of second half B was 6 d). In the third treatment 13 d after transferring plants and after the first inoculation, both sides of five barley plants with split root systems were inoculated. The control treatment consisted of plants that were only inoculated on side B of their split-root system at the second inoculation date.

Root colonization at the time of the second inoculation was determined in a patch of plants inoculated as explained above.

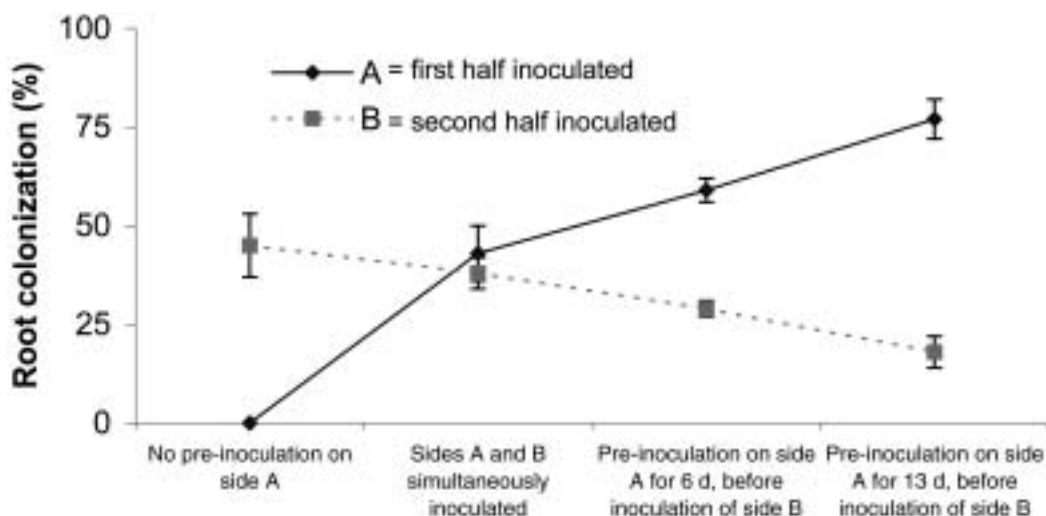
### Level of root colonization

At the time of harvest (12 d after the second inoculation), roots were carefully rinsed with water, cleared by boiling in 10 % KOH, and stained according to the method of Vierheilig et al. (1998) by boiling in a 5 % ink (Shaeffer; black) and usual household vinegar (= 5 % acetic acid) solution. Stained roots were observed with a light microscope to determine the percentage of root colonization according to a modified method of Newman (1966).

The experiment was repeated twice with five replicates per treatment.

## Results

In the first treatment at the day of the second inoculation, the first half of the split-root system (pre-inoculated for 13 d)



**Figure 1.** The effect of pre-inoculation at different time points by AMF on one half of a split-root system (side A), on mycorrhization on the other half (side B) of a split-root system.

showed a root colonization of  $45 \pm 9\%$ . At the end of the experiment (25 d after the first inoculation) root colonization in this treatment was  $77 \pm 5\%$  on the first side of the split-root system and  $18 \pm 4\%$  on the second side (Fig. 1).

In the second treatment at the day of the second inoculation the first half of the split-root system pre-inoculated for 6 d were only slightly mycorrhizal ( $5 \pm 3\%$ ). At the end of the experiment (18 d after the first inoculation) root colonization in this treatment was  $59 \pm 3\%$  on the first side of the split-root system and  $29 \pm 2\%$  on the second side (Fig. 1).

At the end of the experiment, root colonization on the second side was highest in plants not pre-inoculated on the first side (control treatment:  $45 \pm 8\%$ ), with similar levels of root colonization on both sides when both sides were inoculated simultaneously ( $43 \pm 7\%$  and  $38 \pm 4\%$ ). These results indicate a decreasing level of root colonization on the second side of the split-root system when colonization levels increased on the first side (Fig. 1). Results were similar in both experiments. Data from one experiment are shown.

## Discussion

Estimated carbon costs of the mycobiont in a mycorrhizal plant is around 4–20% (Bago et al. 2000, Douds et al. 2000). At first sight the observed suppression of further mycorrhization in already mycorrhizal plants points towards a simple competition for carbohydrates as suggested by Pearson et al. (1993). The fungus first colonizing one side of the split-root system exhibits a strong sink for photoassimilates, thus depriving the second side of the split-root system of carbohydrates necessary for further root colonization. Or to say it differently, the better the first colonizing fungus is established (i.e., a high root colonization), the more carbon strength it exhibits.

This hypothesis seems confirmed when looking at recent results about the carbon sink strength of several AMF (Lerat et al. 2003 a, b). In barley plants grown in a similar split-root system as in our study, with one side colonized by *G. intraradices*, *Gigaspora rosea* or different *G. mosseae* isolates a clear carbon sink effect towards the mycorrhizal side could be observed. Moreover, the sink strength was clearly correlated with the degree of root colonization (Lerat et al. 2003 a). However, there was one exception: the side of a split-root system colonized with the *G. mosseae* isolate BEG 12, the same isolate we used in the present study, in contrast to two other *G. mosseae* isolates (BEG 54 and BEG 55), showed no sink strength, independent of the degree of root colonization and of the host plant (Lerat et al. 2003 a, b). This clearly excludes carbon competition as the regulatory mechanism for the ob-

served suppression of root colonization in already mycorrhizal plants.

Plants colonized by AMF have been shown to exhibit enhanced resistance towards soil-borne pathogenic fungi. Interestingly, only a well established AM symbiosis can protect plants against these pathogens. In several reports the requirement of an extensive root colonization by AMF for an enhanced resistance has been presented (Caron et al. 1986, Cordier et al. 1998) and recently it was suggested that a bioprotective effect depends on a fully developed symbiosis (Slezack et al. 2000). Interestingly, we found a similar effect on mycorrhization. High AMF root colonization levels resulted in a strong suppression of further root colonization, whereas low root colonization levels showed only a reduced suppressional effect on further root colonization.

In our context this could mean that the level of AMF root colonization of a plant not only affects further mycorrhization but also bioprotection against soil-borne pathogens. It is tempting to speculate that similar mechanisms are involved in autoregulation and bioprotection of mycorrhizal plants. Further studies are needed to elucidate the regulatory bases of autoregulation and bioprotection in mycorrhizal plants.

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