



Systemic suppression of mycorrhizal colonization of barley roots already colonized by AM fungi

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Abstract

In a split root system with barley plants, one half of the divided root system had been colonized by the arbuscular mycorrhizal fungi *Glomus mosseae*, *G. intraradices* or *Gigaspora rosea*. After extensive root colonization by the fungi, the other half of the divided root system was inoculated with *G. mosseae*. Prior colonization of one half of the split root system with any of the three fungi resulted in a clear suppression of colonization by *G. mosseae* in the other half of the root system. As carbohydrates are one of the principal elements of plant growth, AM colonization, which is supposed to present a carbon sink, on one half of the split root system, could affect root growth and thus root fresh weight, on the non-mycorrhizal half of the root system. Besides, the improved P-status of mycorrhizal plants has been suggested to affect further AM root colonization. Our experiment showed that neither the root fresh weight, nor the P-concentration differed significantly in split root systems of non-mycorrhizal control plants and in split root systems of half mycorrhizal plants. Thus the suppressional effect in prior AM colonized plants on subsequent AM root colonization seemed not to be linked to a competition of the AM fungi for carbohydrates or an altered P-status. A systemic suppression of further mycorrhization in mycorrhizal roots, which is not AM fungal species or genera specific is discussed. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Roots of about 80% of all land plants are colonized by arbuscular mycorrhizal (AM) fungi. These fungi are a small group of obligately symbiotic fungi in the order *Glomales* (Zygomycotina), which in association with plants, improve plant nutrition and resistance towards a number of pathogens (Dehne, 1982; Linderman, 1994; St.-Arnaud et al., 1995).

It has been observed that after co-inoculation, AM fungal species or genera differ in their ability to form the AM symbiosis (Daft and Hogarth, 1983; Wilson

1984; Hepper et al., 1988; Pearson et al., 1993; 1994). A reduction of colonization of roots by an AM fungus could be observed in roots colonized by another AM fungus. This suppression occurred independently of the AM fungal species or genera tested. However, as different AM fungi were co-inoculated in the same pot, an antagonism between hyphae of fungi in the rhizosphere and in the root could not be excluded. Pearson et al. (1993) used a split root system, inoculating divided parts of a root system simultaneously with two AM fungi, in order to avoid this possible antagonism. Inoculation with *Scutellospora calospora* on one side reduced colonization by *Glomus* sp. on the other side in this system. Pearson et al. (1993) suggested several mechanisms for this antagonistic effect: (i) a competition between different AM fungi for carbon substrates, (ii) a mechanism involving the improved P-

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nutrition of AM colonized plants, or iii) the production of inhibitory compounds in roots of AM colonized plants.

In order to study AM fungal species or genera-specific effects involved, we inoculated roots on one side of a split root system with the AM fungi *Glomus intraradices*, *G. mosseae* or *Gigaspora rosea* and, after an extensive fungal establishment in the root, roots on the other side were inoculated with *G. mosseae*. The colonization of already colonized plants in relation to the root growth and the P-concentration of the roots is discussed.

2. Materials and methods

The three AM fungi *Glomus mosseae* (Nicolson and Gerdemann) Gerd. and Trappe (BEG 12; La Banque Européenne des Glomales; International Institute of Biotechnology; Kent; GB), *G. intraradices* Smith and Schenck (DAOM 197198; Department of Agriculture, Ottawa, Canada) and a recently newly classified *Gigaspora rosea* Nicolson and Schenck (Bago et al., 1998), which was formerly wrongly classified as *Gigaspora margarita* Becker and Hall (DAOM 194757) were used.

Barley (*Hordeum vulgare* L. cv. Salome) seeds were surface sterilized in 50% commercial bleach for 5 min, rinsed several times in tap water and germinated in

vermiculite. After 5 d 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 dia of approximately 2–5 mm; Applied Industrial Materials, Corp.; Buffalo Grove, IL, USA) and soil (vol:vol:vol/2:2:1) into the test plant compartments B and C (Fig. 1). Plants were grown in a growth chamber (day/night cycle: 16 h; 23°C/8 h; 19°C; rel. humidity 50%) in a modified compartment system (Figs. 1 and 2) of Wyss et al. (1991). The system (Fig. 1) consists of unpartitioned inoculum compartments (A+D) and partitioned test plant compartments (B+C). The test plant compartments (B+C) are divided into five subcompartment units (3.3 × 10 × 2 cm). The inoculum compartments (A+D) (20 × 10 × 2 cm) are equipped on the side facing the test plant compartments with a nylon screen (60 µm mesh size) and contain beans (*Phaseolus vulgaris* L. cv. Sun Gold) with the inoculum of the different AM fungi or mock inoculum. Inoculum compartments were prepared by growing beans in the silicate sand, TurFace[®], soil substrate described above in the presence of the spores of the AM fungi. The AM symbiosis was well established in the inoculum compartments after 1 month. Control mock inoculum compartments were prepared identically as fungal inoculum compartments, except in absence of the fungi and with a water filtrate of a mixture of the three inocula (McAllister et al., 1997).

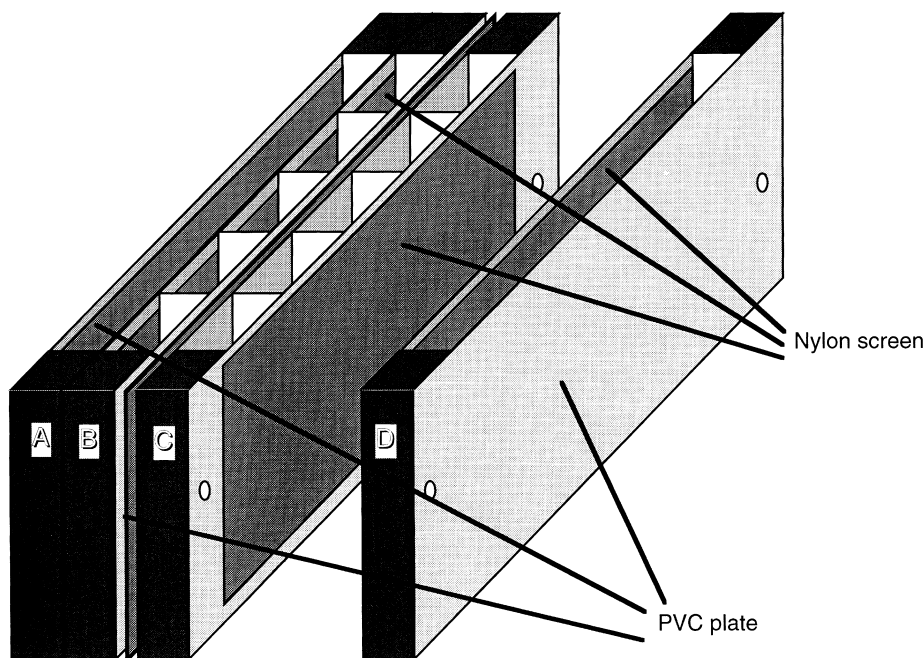


Fig. 1. The compartment system consists of two inoculum compartments (Comp. A + D) and two test-plant compartments (Comp. B + C). The test plant compartments are divided into five subcompartment units. Compartment A and B and compartment C and D are separated by nylon screens, which can be penetrated by AM fungal hyphae, but not by roots. Compartment B and C are separated by a PVC-plate, therefore hyphae and roots cannot pass.

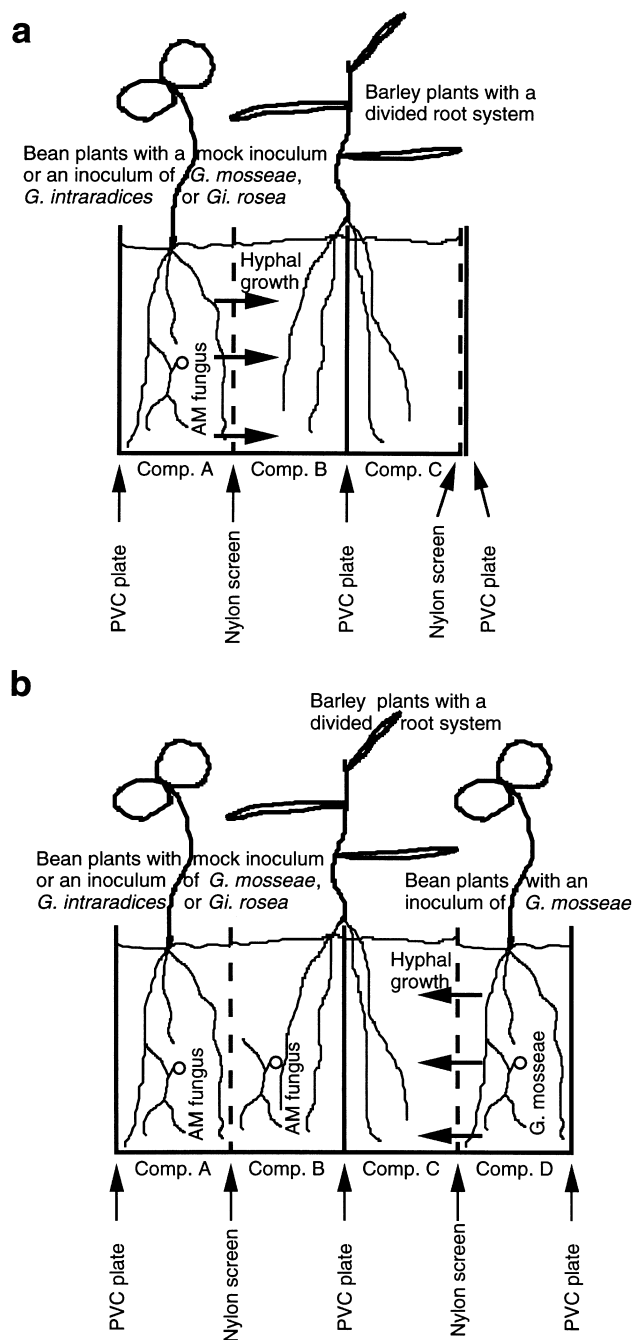


Fig. 2. The compartment system in combination with a split root system. (a) Once compartment A with an inoculum of *Glomus intraradices*, *Glomus mosseae*, or *Gigaspora rosea* is joined with compartment B, hyphae pass to compartment B and colonize the barley roots in compartment B, however, barley roots in compartment C are not colonized. (b) After an extensive root colonization in compartment B (12 d after joining compartment A with compartment B), compartment D with an inoculum of *G. mosseae* is joined with compartment C. Hyphae of *G. mosseae* from compartment D pass through the nylon screen to compartment C, colonizing the as yet non-colonized barley roots.

The two test plant compartments B and C are glued together, but separated by an impermeable PVC plate ($20 \times 10 \times 0.1$ cm); thus no hyphae could pass from compartment B to compartment C (see Fig. 1). The root system of the barley plants was divided and planted in compartment B and compartment C (Fig. 2). The test plant compartments (B+C) are equipped on the side facing the inoculum compartments with a nylon screen (Fig. 1). The nylon screens between the compartments can be penetrated by fungal hyphae but not by roots. Attachment of the inoculum compartments to the test plant compartments (Figs. 1 and 2) results in a fast root colonization of the plants in the test plant compartments. Nylon screens were covered by PVC plates ($20 \times 10 \times 0.1$ cm) to prevent desiccation before joining the test plant compartments with an inoculum compartment.

Twelve days after joining the inoculum compartment A (with the AM fungi *G. mosseae*, *G. intraradices*, or *Gi. rosea*) with the test plant compartment B, containing one half of the split root system of the barley plants (Fig. 2a), inoculum compartment D with an inoculum of *G. mosseae* was joined to compartment C, with the other half of the split root system of the barley plants (Fig. 2b). Twelve days after the first inoculation (inoculation with *G. mosseae*, *G. intraradices*, or *Gi. rosea*) and 9 d after the second inoculation (inoculation with *G. mosseae*), five plants/treatment were harvested. Thereafter roots were rinsed with tap water and the root fresh weight was determined. Roots were stained according to the method of Vierheilg et al. (1998), by clearing in boiling 10% KOH for 3 min and by staining for 3 min in a boiling ink (Shaeffer; black)/usual household vinegar (= 5% acetic acid) solution. The method was slightly modified by using a 3% instead of a 5% ink solution, which gave similar staining results. Roots were destained for 1 h in tap water acidified with a few drops of vinegar. The percentage of root colonization was determined by quantifying the presence or absence of fungal structures within roots at 100 intercepts (Newman, 1966).

For the analysis of the P-concentration, dried roots (30 h; 65°C) were digested (Parkinson and Allen, 1975) and P was determined by a Perkin Elmer Emission Plasma 40 Spectrometer. All measurements were performed with five replicates per treatment. Means and standard error of means are given.

3. Results

After 12 d barley roots in compartment B showed a colonization rate of 47% with *G. mosseae*, 77% with *G. intraradices* and 58% with *Gi. rosea* (Table 1). Nine days after inoculating barley plants in compartment C with *G. mosseae*, control plants with no prior coloniza-

Table 1

Effect of prior colonization with AM-fungi on root colonization by *G. mosseae* in a split root system. The root system was divided in two different compartments (Comp. B, Comp. C). Twelve days after inoculating Comp. B with different AM-fungi (*G. mosseae*, *G. intraradices* or *Gi. rosea*), Comp. C was inoculated for 9 d with *G. mosseae*. Root colonization was determined as percentage of colonized root length after 12 and 12+9 d^a

12 d after inoculation of compartment B							
Plants with divided root systems		Plants with divided root systems		Plants with divided root systems		Plants with divided root systems	
Comp. B	Comp. C	Comp. B	Comp. C	Comp. B	Comp. C	Comp. B	Comp. C
No inoculation 0	No inoculation 0	<i>G. mosseae</i> 47 ± 7	No inoculation 0	<i>G. intraradices</i> 77 ± 11	No inoculation 0	<i>Gi. rosea</i> 58 ± 9	No inoculation 0
9 d after inoculation of compartment C							
No inoculation 0	<i>G. mosseae</i> 59 ± 12	<i>G. mosseae</i> 69 ± 5	<i>G. mosseae</i> 6 ± 4	<i>G. intraradices</i> 79 ± 5	<i>G. mosseae</i> 8 ± 4	<i>Gi. rosea</i> 90 ± 5	<i>G. mosseae</i> 8 ± 7

^a Data represent mean ± S.E. ($n = 5$).

tion were highly colonized (59%), whereas all plants with roots already colonized by different AM fungi in compartment B, showed a similar low root colonization in compartment C (6–8%) (Table 1). Arbuscules were visible in all plants colonized by AM fungi.

Twelve days after the first inoculation, the root fresh weight of the plants with one half of the split root system colonized by the different AM fungi (Comp. B) was reduced compared to the split root system of non-mycorrhizal control plants, however, this reduction was not statistically significant (Table 2). The non-mycorrhizal half of a split root system (Comp. C) of mycorrhizal plants showed no significant differences of root fresh weight compared to the split root system of a non-mycorrhizal control plants. The root fresh weight in the non-mycorrhizal half of plants colonized by *G. mosseae* or *G. intraradices* was significantly higher compared to the root fresh weight of the mycorrhizal half of the split root system of these plants.

The P-concentration (12 d after the first inoculation) was similar in roots of non-mycorrhizal control plants and in plants with half of the split root system colonized by *G. mosseae* or *G. intraradices* (Table 3). The

root P-concentration of the split root system colonized by *Gi. rosea* was significantly enhanced on the mycorrhizal half compared to non-mycorrhizal control plants, whereas the non-mycorrhizal half showed no significant difference. Mycorrhizal and non-mycorrhizal roots of split root systems with the mycorrhizal side colonized by *Gi. rosea* showed an enhanced P-concentration compared to mycorrhizal and non-mycorrhizal roots of split root systems with the mycorrhizal side colonized by *G. mosseae* or *G. intraradices*.

4. Discussion

Pearson et al. (1993) simultaneously inoculated split clover root systems with *S. calospora* and *Glomus* sp. and observed a weak reduction in the colonization by *Glomus* sp. and suggested a competition for carbohydrates between the two fungi. They concluded *S. calospora* had a greater capacity to obtain carbon substrates from the host than *Glomus* sp. and thereby decreased the availability of carbohydrates, thus reducing the root colonization by *Glomus* sp. Our observation, showing that prior colonization by *G. mosseae*

Table 2

Effect of colonization by *G. mosseae*, *G. intraradices* or *Gi. rosea* on the root fresh weight (mg) of barley in a split root system. The root system was divided in two different compartments (Comp. B, Comp. C)^a

12 d after inoculation of compartment B							
Plants with divided root systems		Plants with divided root systems		Plants with divided root systems		Plants with divided root systems	
Comp. B	Comp. C	Comp. B	Comp. C	Comp. B	Comp. C	Comp. B	Comp. C
No inoculation 430 ± 60	No inoculation 450 ± 40	<i>G. mosseae</i> 350 ± 60	No inoculation 530 ± 100	<i>G. intraradices</i> 370 ± 80	No inoculation 530 ± 50	<i>Gi. rosea</i> 370 ± 30	No inoculation 430 ± 30

^a Data represent mean ± S.E. ($n = 5$).

also reduces subsequent colonization by *G. mosseae* and this reduction is similar with *G. intraradices* and *Gi. rosea*, makes it rather unlikely that the different competitiveness of different AM fungi for carbohydrates is the responsible mechanism for the reduced colonization. A different competitiveness of the three AM fungi for carbohydrates should result in different degrees of suppression of the later colonizing *G. mosseae*. However, the observed reduction of subsequent root colonization was independent of the prior colonizing AM fungal species or genera. Our results rather point to the hypothesis, that a faster or already established AM fungal colonizer has an advantage over a slower or later colonizer, probably acting as a carbon sink and thus depriving the slower/late colonizer from part of its nutrient source.

Carbohydrates are one of the principal elements of plant growth. Low concentrations of carbohydrates e.g. under low light conditions, result in reduced plant growth and also in reduced root colonization by AM fungi (Hayman, 1982; Smith and Read, 1997). In split root systems with one half of the roots colonized by an AM fungus and the other half non-colonized more photosynthate is transferred to the mycorrhizal side indicating a carbon sink (Douds et al., 1988; Wang et al., 1989). However, in the experiment of Douds et al. (1988) the carbohydrate transfer rate to mycorrhizal roots differed only slightly (3 to 4% higher) from the transfer rate to the non-mycorrhizal roots. Unfortunately from those experiments data about the root growth of the mycorrhizal side or the non-mycorrhizal side of the split root system and of non-mycorrhizal control plants with a split root system are not available. A strong carbon sink on the mycorrhizal side of a split root system should result in a reduced root growth on the non-mycorrhizal side; however, in our experiment the non-mycorrhizal half of the root system of mycorrhizal barley plants had a similar root fresh weight as roots from non-mycorrhizal control plants. As there were apparently sufficient carbohydrates in the non-mycorrhizal half of the mycorrhizal plants, to sustain root growth as in non-mycorrhizal plants, carbohydrates should not be the limiting factor for

root colonization. This makes it rather unlikely that the observed reduction of root colonization is due to a competition for carbohydrates between an already established AM fungus and a later colonizing AM fungus.

The improved P-status of mycorrhizal plants has been suggested as another possible factor affecting root colonization. In many studies about the effect of P on AM root colonization, P was applied directly to the soil, thus a direct P effect and a plant mediated P-effect on AM fungi could not be discriminated (Daft and Nicolson, 1969; Mosse, 1973; Sanders and Tinker, 1973 and many reports thereafter). To study exclusively the plant mediated P-effect on root colonization, split root systems were used (Menge et al., 1978; Koide and Li, 1990; Thomson et al., 1991) and the P-concentration in the roots on both sides and its effect on root colonization was determined (Thomson et al., 1991). When high concentrations of P were applied on one side, a clear negative effect on root colonization on the other side was observed (Menge et al., 1978; Koide and Li, 1990; Thomson et al., 1991). Plants colonized on one half of the split root system by *Gi. rosea*, in our experiment, showed an increased P-concentration in the mycorrhizal and the non-mycorrhizal half of the root system and subsequent colonization by *G. mosseae* was reduced. These observations seemed to point toward a P-mediated mechanism regulating further root colonization. However, the data obtained with *G. mosseae* or *G. intraradices* colonized plants gave a different picture. Mycorrhizal and non-mycorrhizal halves of split root systems of plants colonized by one of the two fungi had no significant differences in P-concentration compared to split root systems of non-mycorrhizal control plants; however, they showed a similar reduction of root colonization in Compartment C as *Gi. rosea* colonized plants. These results indicate that in our experimental conditions, a factor which is not linked to the P-status of the plant regulates further AM root colonization.

To explain the reduced further root colonization of AM colonized plants a third hypothesis was brought forward by Pearson et al. (1993): the production of in-

Table 3

Effect of colonization by *G. mosseae*, *G. intraradices* or *Gi. rosea* on the P-concentration in roots in a split root system. The root system was divided in two different compartments (Comp. B, Comp. C). The P-content of dried roots is given as $\mu\text{g g}^{-1}$ ^a

12 d after inoculation of compartment B							
Plants with divided root systems		Plants with divided root systems		Plants with divided root systems		Plants with divided root systems	
Comp. B	Comp. C	Comp. B	Comp. C	Comp. B	Comp. C	Comp. B	Comp. C
No inoculation	No inoculation	<i>G. mosseae</i>	No inoculation	<i>G. intraradices</i>	No inoculation	<i>Gi. rosea</i>	No inoculation
3509 ± 345	3477 ± 268	2903 ± 277	3113 ± 320	3096 ± 174	3092 ± 240	4237 ± 201	3909 ± 342

^a Data represent mean ± S.E. ($n = 5$).

hibitory compounds in roots of AM colonized plants. The suppression of the mycorrhizospheric mycoflora has been attributed to physiological changes in mycorrhizal roots, possibly resulting in the release of mycotoxic compounds (Bansal and Mukerji, 1994). Moreover, Pinior et al. (1999) showed that root exudates of mycorrhizal plants exhibit an inhibitory effect on root colonization by AM fungi. Our results, showing a reduced spread of AM colonization in the non-mycorrhizal roots of mycorrhizal plants, could point toward a systemic release of mycotoxic compounds from mycorrhizal and non-mycorrhizal roots of AM colonized plants. There is some information about systemic plant defense mechanisms involved in the enhanced resistance of mycorrhizal plants against pathogens (Davis and Menge, 1980; Rosendahl, 1985; Cordier et al., 1998), however, until now no systemic mechanisms have been suggested to be involved in the regulation of further colonization by AM fungi in mycorrhizal plants.

Grandmaison et al. (1993) showed that AM fungi from different species, apart from inducing similar amounts of some secondary plant compounds, also induce different amounts of other compounds in roots. Therefore it was tempting to speculate whether roots colonized by one AM fungal species or genera, accumulate compounds that are antagonistic to the development of other AM fungal species or genera. However, our results show a general, AM fungal species or genera non-specific suppression of further root colonization. Pinior et al. (1999) found a similar non-specificity with root exudates: Root exudates from plants colonized by the AM fungi *G. mosseae*, *G. intraradices* or *Gi. rosea* exhibited the same inhibitory effect on root colonization by *G. mosseae*.

To summarize, our results point toward a systemic mechanism suppressing further root colonization by AM fungi in mycorrhizal plants. Further studies are needed to elucidate (i) whether an altered root exudation is involved in the observed suppression and whether it shares features with (ii) the systemic resistance of mycorrhizal plants towards pathogens or (iii) with SAR (systemic acquired resistance) in non-mycorrhizal plant-pathogen systems (recently reviewed by Sticher et al., 1997).

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