Bioprotection against *Gaeumannomyces graminis* in barley – a comparison between arbuscular mycorrhizal fungi

V. Castellanos-Morales[1,3], R. Cárdenas-Navarro[2], J.M. García-Garrido[3], A. Illana[3], J.A. Ocampo[3], S. Steinkellner[4], H. Vierheilig[3]

[1]*Corporativo de Desarrollo Sustentable (COSUSTENTA), Circuito Parque Industrial 252, Colonia Ciudad Industrial, Morelia Michoacán, México*
[2]*Instituto de Investigaciones Agropecuarias y Forestales (IIAF), Carretera Morelia-Zinápecuaro, Tarimbaro Michoacán, México*
[3]*Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín (EEZ), CSIC, Granada, Spain*
[4]*Division of Plant Protection (DCS), University of Natural Resources and Life Sciences, Vienna, Austria*

ABSTRACT

Gaeumannomyces graminis var. *tritici* causes take-all disease, the most important root disease of cereal plants. Cereal plants are able to form a symbiotic association with soil-borne arbuscular mycorrhizal fungi which can provide bioprotection against soil-borne fungal pathogens. However, the bioprotective effect of arbuscular mycorrhizal fungi against soil-borne fungal pathogens might vary. In the present study we tested the systemic bioprotective effect of the arbuscular mycorrhizal fungi *Glomus mosseae*, *Glomus intraradices* and *Gigaspora rosea* against the soil-borne fungal pathogen *Gaeumannomyces graminis* var. *tritici* in a barley split-root system. *Glomus intraradices*, *Glomus mosseae* and *Gigaspora rosea* colonized the split-root system of barley plants at different levels; however, all arbuscular mycorrhizal fungi clearly reduced the level of root lesions due to the pathogen *Gaeumannomyces graminis*. Our data indicate that some arbuscular mycorrhizal fungi need high root colonization rates to protect plants against fungal pathogens, whereas others act already at low root colonization rates.

Keywords: soil-borne fungi; take-all diseases; *Gigaspora rosea*; *Glomus* sp; *Hordeum vulgare*

Gaeumannomyces graminis var. *tritici* (Ggt), a soil-borne fungal pathogen, causes take-all disease, one of the most important root disease of cereal plants such as barley, wheat and rye (Mathre 1992). These cereal plants, like 80% of all land plants, are able to form a symbiotic association with the soil-borne arbuscular mycorrhizal fungi (AMF) which can provide bioprotection against soil-borne fungal pathogens (St-Arnaud and Vujanovic 2007, Vierheilig et al. 2008).

Several reports are available on a bioprotective effect of AMF against Ggt. In wheat and barley it was reported that AM root colonization locally and systemically reduces root lesions caused by Ggt (Graham and Menge 1982, Khaosaad et al. 2007, Vierheilig et al. 2008, Castellanos-Morales et al. 2011) and diminishes a negative effect of Ggt on root growth (Graham and Menge 1982, Ksiezniak et al. 2001, Khaosaad et al. 2007, Vierheilig et al. 2008, Castellanos-Morales et al. 2011).

Several variables seem to affect the bioprotective effect of mycorrhization. Bioprotection through mycorrhization seems to depend on the plant genotype, the degree of AM root colonization and on the root-colonizing AMF. When working with different strawberry and potato genotypes bioprotection through mycorrhization against soil-borne pathogens varied (Mark and Cassells 1996, Yao et al. 2002). Most recently we found something similar for Ggt, when working with

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split-root systems of barley. The systemic bioprotective effect of AM root colonization against Ggt varied with the barley variety (Castellanos-Morales et al. 2011).

The degree of root colonization seems to be another decisive factor for bioprotection through AMF. Low AM colonization levels were reported to show no bioprotective effect, whereas after a critical level of AM root colonization bioprotection is observed (Cordier et al. 1998). A similar effect was reported with Ggt. Only high levels of AM root colonization provided local and systemic bioprotection against Ggt (Graham and Menge 1982, Khaosaad et al. 2007, Vierheilig et al. 2008).

However, mycorrhizal bioprotection not only seems to depend on the plant variety and on the degree of AM root colonization but also on the root-colonizing AMF with some AMF providing bioprotection and others not (Matsubara et al. 1995, 2000, Pozo et al. 2002, Yao et al. 2002, Carlsen et al. 2008).

The objective of the present study was to find out whether the systemic bioprotective effect of mycorrhization against the soil-borne fungal pathogen *Gaeumannomyces graminis* var. *tritici* (Ggt) varies between the AMF *Glomus mosseae*, *Glomus intraradices* and *Gigaspora rosea*.

MATERIAL AND METHODS

Plant and fungal material. The barley (*Hordeum vulgare* L.) variety Nürnberg was used. *Gaeumannomyces graminis* var. *tritici* was obtained from Centraalbureau voor Schimmelcultures, the Netherlands (CBS 541.86). The following AMF were tested: *Glomus mosseae* (Nicolson & Gerdemann) Gerd. & Trappe (BEG 12; International Bank for the Glomeromycota); *Glomus intraradices* Smith & Schenck (DAOM 197198); *Gigaspora rosea* Nicolson & Schenck (DAOM 194757).

Growth conditions. Barley seeds were surface-sterilized by soaking in sodium hypochlorite: water (1:1 v/v) solution for 5 min, rinsed with tap water and seeded in sterilized vermiculite. After 6 days the barley plants were transferred to a split-root system consisting of two compartment units, each containing half of the barley root system. The two compartment units were separated by a PVC screen, impermeable for molecules, roots or hyphae. The compartments were filled with an autoclaved mixture (20 min, 121°C) of silicate sand and soil (3:2 v/v). Each compartment box comprises five plants (Vierheilig et al. 2000, Khaosaad et al. 2007). Experiments were performed under natural conditions in a greenhouse. Plants were watered 4 times a week with tap water.

AMF and Ggt inoculation. The experiments included the following treatments: (i) plants were not inoculated on the first side of the split-root system with one of the AMF, but the second side of the split-root system was inoculated with Ggt (Ggt+/AMF–); (ii) plants were inoculated on the first side of the split-root system with one of the AMF and with Ggt on the other side of the split-root system (Ggt+/*G. intraradices*–; Ggt+/*G. mosseae*+; Ggt+/*Gi. rosea*+). In the control treatment plants were not inoculated at each side of the split root system (Ggt–/AMF–). Figure 1 depicts the chronological process of setting up the experiments.

The inoculation of the half of the split barley root system with the AMF was done 3 days after transplanting the barley plants into the split-root system. The outer side of each split-root compartment was equipped with a nylon screen (30 µm mesh). This allows the AM hyphae (but not the plant roots) to penetrate the compartment. Moreover the outer side of this split-root compartment was joined with an inoculum compartment containing plants of *Sorghum vulgare* previously colonized with *G. mosseae*, *G. intraradices* or *Gi. rosea*. This inoculum compartment was also equipped with a nylon screen (30 µm mesh), allowing the hyphae from the inoculum compartment to colonize the roots on one side of the split-root compartment.

For the Ggt inoculation the pathogen was cultured on Potato Dextrose agar (39 g/L) (Fluka, Steinheim, Germany) in Petri dishes at 25°C for 7 days in the dark. Vigorously growing mycelium from the edge of the colony agar discs were removed and transferred to Erlenmeyer flasks containing 40 g of autoclaved (20 min, 121°C) barley seeds. These flasks were incubated at 28°C for 12 days in the dark. 6 g of the obtained inoculum was applied per split-root/plant according to the method described by Mathre (1992).

In order to obtain a homogenous Ggt infection 7 days prior to Ggt inoculation the whole compartment system was inclined (45° angle) in such a way that the roots of the side of the split-root system, which were later Ggt inoculated, grew downwards onto a PVC screen. Thus, after 7 days the PVC screen could be removed and the inoculum (6 g inoculated barley seeds/plant) could be applied directly the roots.

Determination of AMF colonization and severity caused by Ggt. AMF root colonization was determined at the time of Ggt inoculation (42 days after AMF inoculation) in 5 additional plants per treatment. At the time of harvest (28 days after

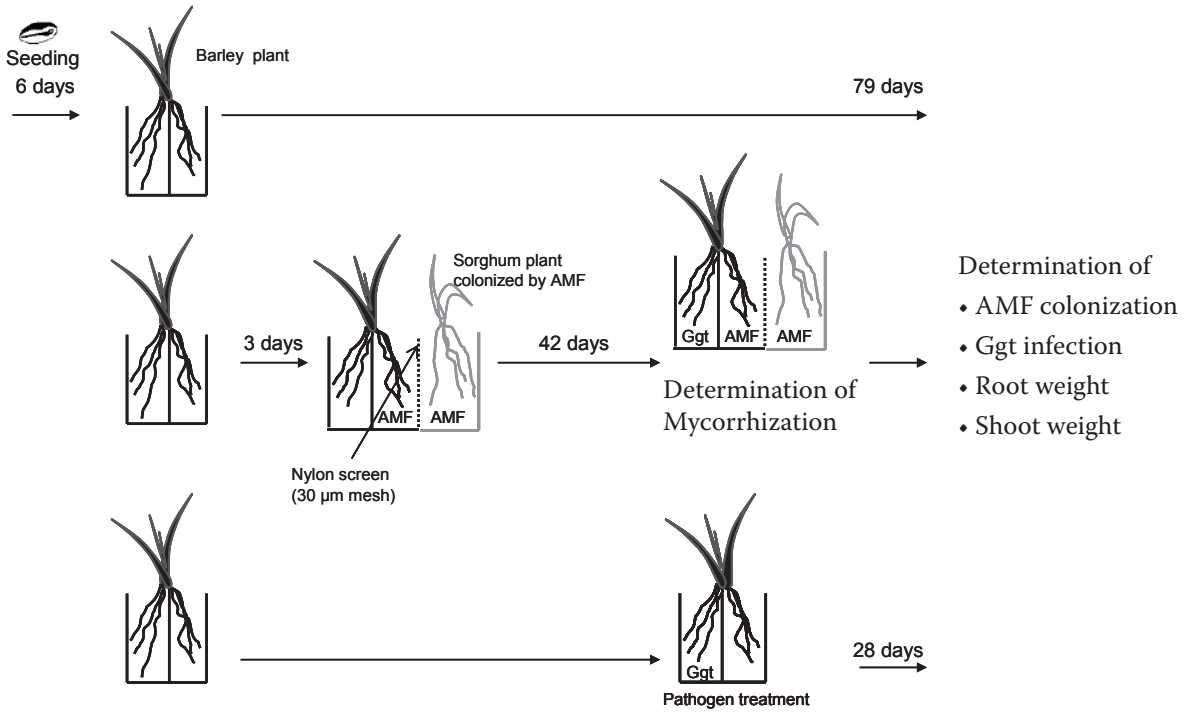

Figure 1. Chronological process of setting up the experiments

Ggt inoculation; total plant age 79 days) the plants were removed from the substrate by gently washing off the roots with tap water. Subsequently, the root system was separated from the shoots and the fresh weights of the roots and shoots were determined. In fresh roots the percentage of Ggt infection was determined by scoring visibly lesioned roots. Whitish roots were scored as non-lesioned, whereas yellow to dark brown root sections were scored as lesioned (Graham and Menge 1982). The percentage of roots lesioned by Ggt was determined according to a modified method of Newman (1966). For the determination of AM root colonization fresh roots were cleared (7 min boiling in 10% KOH) and stained by boiling in a solution of 5% ink (Sheaffer black) in household vinegar (5% acetic acid) according to the method of Vierheilig et al. (1998). Thereafter, the AMF root colonization was determined microscopically according to the method of Newman (1966).

Statistical analysis. Statistical analyses were performed using SYSTAT for Windows, version 11.0. Variance comparisons were done using the Fisher's least significant difference test ($P < 0.05$, $n = 5$).

RESULTS

At the time point of Ggt inoculation AM root colonization was highest with *G. intraradices* (around 50%), followed by *G. mosseae* (around 40%), whereas root colonization with *Gi. rosea* was under 3%) (Figure 2). At the end of the experiments AM root colonization was again highest with *G. intraradices* (around 70%), followed by *G. mosseae* (around 45%), whereas root colonization with *Gi. rosea* was still low (7%) (Figure 2).

In the treatment without AMF (Ggt+/AMF–) inoculation with Ggt resulted in a clear increase of lesioned roots (Figure 3). In all treatments with an AMF on one side of the split root system, in the roots on the other side of the split root system inoculated with Ggt (Ggt+), the percentage of lesioned roots was similar as in the control without AMF and Ggt (Ggt–/AMF–), showing that AM root colonization with each of the tested AMF reduces lesioning of the roots (Figure 3).

In the treatments without AMF (Ggt+/AMF–) and with an AMF on the first side of the split root system, inoculation with Ggt (Ggt+) resulted in a clear decrease of the root fresh weight on the Ggt-inoculated side of the split root system compared to the control treatment without AMF and Ggt (Ggt–/AMF–) (Figure 4). Inoculation with *G. intraradices* on the first side of the split root system without Ggt-inoculation on the second side of the split root system reduced the root fresh weight on the second side of the split root system to a similar extent as with Ggt-inoculation on the second side of the split root system. The

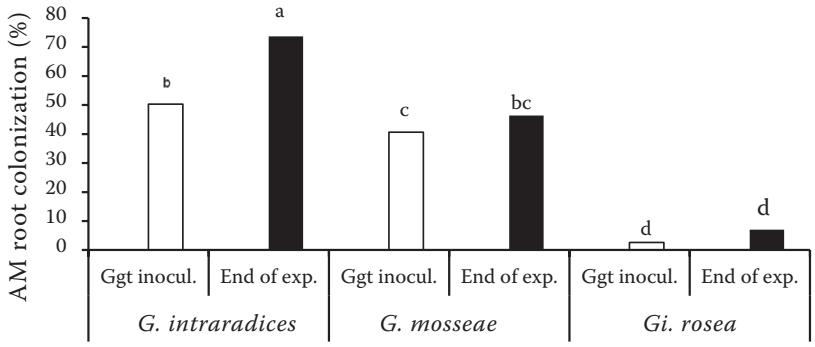

Figure 2. Root colonization by arbuscular mycorrhiza fungi at the time of Ggt inoculation and at the end of the experiment. Columns followed by different letter are significantly different according to Fisher's least significant difference test ($P < 0.05$, $n = 5$). Systat for Windows vers. 11.0

treatment with *G. mosseae* and *Gi. rosea* did not show such effect (Figure 4).

In the treatment without AMF (Ggt+/AMF–) inoculation with Ggt showed no effect on the shoot fresh weight compared to the control treatment (Ggt–/AMF–) (Figure 5). Inoculation with *G. intraradices* reduced the shoot fresh weight in the treatment without Ggt-inoculation (Ggt–) and this reduction was even more pronounced when AMF and Ggt were inoculated (Ggt+). Inoculation with *G. mosseae* resulted in the treatment with and without Ggt inoculation in similar shoot fresh weights as in the control treatment without AMF and Ggt inoculation (Ggt–/AMF–). A significant increase of the shoot fresh weight compared to the control plants (AMF–/Ggt–) could be observed in both treatments (Ggt– and Ggt+) inoculated with *Gi. rosea* (Figure 5).

DISCUSSION

There is abundant information on the bioprotective effect of root colonization by AMF against soil-borne pathogens (St-Arnaud and Vujanovic 2007), however, in most studies one AMF was tested and thus, a comparison of the bioprotective effect between AMF is difficult. Moreover, in most studies *Glomus* species were tested and only scarce data are available on the bioprotective effect of other AM genera (St-Arnaud and Vujanovic 2007, Vierheilig et al. 2008).

Testing several AMF Pozo et al. (2002) reported a bio-protective effect of *G. mosseae* against *Phytophthora parasitica* in tomato, whereas *G. intraradices* did not show such effect. In egg plants Matsubara et al. (1995) showed that *G. etunicatum* was more efficient against verticillium wilt than *Gi. margarita*. In contrast, Matsubara et al. (2000) showed in asparagus that *Gi. margarita* was more efficient against the pathogen *Helicobasidium mompa* than *Glomus* species. In one study with white clover, *G. mosseae* completely prevented infection by *Pythium ultimum* whereas *G. claroideum* only reduced infection by the pathogen (Carlsen et al. 2008).

In our study, when looking at the lesioned roots due to Ggt, we found that all 3 AMF tested (*G. intraradices*, *G. mosseae* and *Gi. rosea)* acted similarly by reducing the number of lesioned roots. Recently, in a similar split-root system with *G. mosseae* pre-inoculated on one side and Ggt on the other side it was suggested that mycorrhization only affects Ggt-infection above a certain degree of AM root colonization, whereas a low AM root colonization shows no effect (Khaosaad et al. 2007). In our study with *G. intraradices* and *G. mosseae* we found that a high AM colonization results in a decrease of lesioned roots. With *Gi. rosea* the picture was different. Roots were only colonized by the AMF at very low levels, but independently

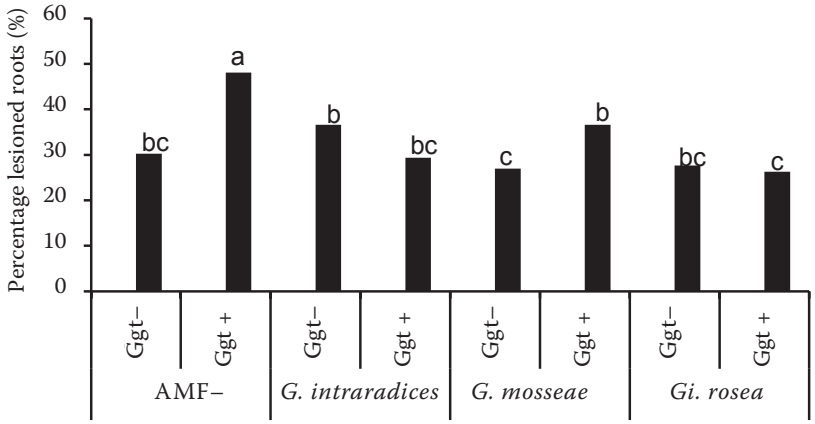

Figure 3. Lesioned roots on the second side of the split-root system inoculated or non-inoculated with Ggt, when the first side of the split-root system was inoculated or non-inoculated with an AMF. Columns followed by different letter are significantly different according to Fisher's least significant difference test ($P < 0.05$, $n = 5$). Systat for Windows vers. 11.0

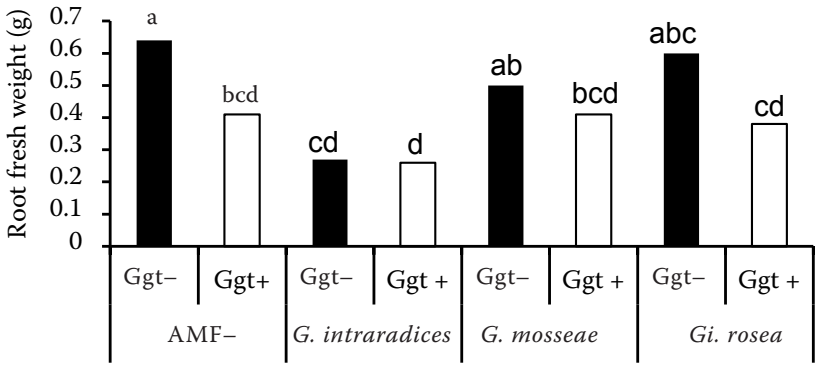

Figure 4. Root fresh weight on the second side of the split-root system inoculated or non-inoculated with Ggt, when the first side of the split-root system was inoculated or non-inoculated with an AMF. Columns followed by different letter are significantly different according to Fisher's least significant difference test ($P < 0.05$, $n = 5$). Systat for Windows vers. 11.0

of this low root colonization, the percentage of lesioned roots was clearly decreased. The positive effect on root lesioning with *Gi. rosea* with low AM root colonization was even more pronounced than with *G. mosseae* with a high AM root colonization. This could be explained by a varying bioprotective effect of different AMF. To protect plants some AMF might need high colonization rates whereas other AMF do not. In several studies with *G. intraradices* it was reported that independently of the level of AM root colonization a disease reduction could be observed (Caron et al. 1986, St-Arnaud et al. 1994, 1997), whereas with *G. mosseae* a local and systemic protective effect against Ggt could be only observed at high AM root colonization levels (Khaosaad et al. 2007, Vierheilig et al. 2008).

In our experiment Ggt inoculation not only affected the number of lesioned roots but also the root fresh weight. When Ggt was applied the root fresh weight was clearly reduced on the side of the split-root system where Ggt was inoculated. However, the root fresh weight of the side of the split-root system where Ggt was inoculated showed no difference in plants without AM inoculation or in plants inoculated with one of the 3 AMF, indicating that mycorrhization had no effect on the root growth in presence of Ggt.

The picture was different when looking at the shoot fresh weight. Ggt-inoculated plants without AMF showed the same shoot growth as plants without Ggt. This means that in our experimental conditions Ggt did not affect the shoot growth. However, we found that the shoot growth was affected by the root colonizing AMF. Plants colonized by *G. intraradices* showed a drastically reduced shoot fresh weight without Ggt and this effect was even more pronounced when Ggt was inoculated. A similar shoot growth depression with *G. intraradices* was reported before with tomato and *G. intraradices* was suggested to be a strong carbon sink, thus resulting in reduced growth (Pozo et al. 2002). This hypothesis was confirmed in split-root systems of barley showing that at a high degree of root colonization *G. intraradices* acts as a strong carbon sink (Lerat et al. 2003). Interestingly we observed the highest shoot growth with *Gi. rosea*, which showed the lowest levels of AM root colonization, indicating that at least with *Gi. rosea* the levels of AM root colonization is not necessarily a parameter for a successful AM root colonization in terms of plant growth.

To summarize, in our study despite of the different degree to which they colonized the split-root system of the barley plants *G. intraradices*, *G. mosseae* and *Gi. rosea* did not differ in their positive effect on Ggt-infection. Possibly some AMF need high root colonization rates to protect plants against fungal pathogens, whereas other AMF act already at low root colonization rates. In further works it would be of interest to study how high root colonization rates of *Gi. rosea* or other *Gigaspora* species act on pathogens. Moreover, molecular genetic tools will

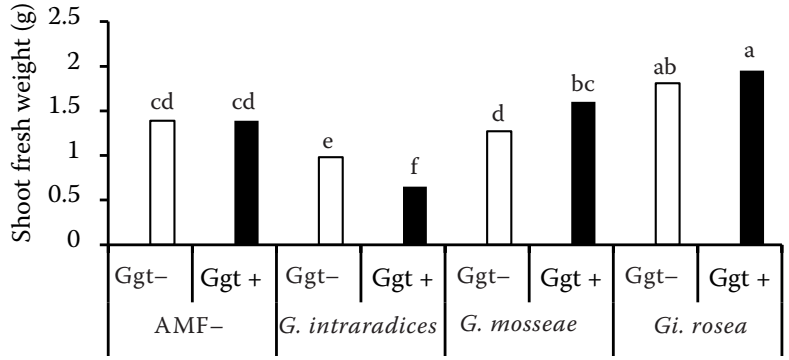

Figure 5. Shoot fresh weight when one side of the split-root system was first inoculated or non-inoculated with an AMF and thereafter the second side of the split-root system was inoculated or non-inoculated with Ggt. Columns followed by different letter are significantly different according to Fisher's least significant difference test ($P < 0.05$, $n = 5$). Systat for Windows vers. 11.0

be advantageous for the elucidation of the observed controversial fungal effects.

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In memoriam of Horst Vierheilig.

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Corresponding author:

Ao. Prof. DI Dr. Siegrid Steinkellner, University of Natural Resources and Life Sciences, Vienna, Division of Plant Protection, Peter Jordan-Strasse 82, 1190 Vienna, Austria
phone: + 43 476 543 352, fax: + 43 476 543 359, e-mail: siegrid.steinkellner@boku.ac.at