

Take-all disease is systemically reduced in roots of mycorrhizal barley plants

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Abstract

The systemic effect of root colonization by the arbuscular mycorrhizal fungus *Glomus mosseae* on infection of barley by *Gaeumannomyces graminis* var. *tritici* (Ggt) was studied. In split-root systems of barley one side was inoculated with *G. mosseae* and the other side was inoculated with Ggt.

Root infection by Ggt was systemically reduced when barley plants showed high degrees of mycorrhizal root colonization, whereas a low mycorrhizal root colonization exhibited no effect on Ggt infection. Our results show a clear systemic bioprotective effect depending on the degree of root colonization by the mycorrhizal fungus. At a higher mycorrhizal colonization rate the concentration of salicylic acid (SA) was increased in roots colonized by the mycorrhizal fungus but no systemic increase of SA could be measured in non-mycorrhizal roots of mycorrhizal plants, indicating that the systemic bioprotective effect against Ggt is not mediated by salicylic acid. © 2006 Elsevier Ltd. All rights reserved.

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1. Introduction

Arbuscular mycorrhizal fungi (AMF) colonize roots of most plant species. AMF and plants live in a symbiotic relationship where both partners derive benefits from the association. Mycorrhizal plants show an improved nutrient status, mainly phosphorus (P), whereas the AMF is provided by the plant with carbohydrates (Smith and Read, 1997).

Mycorrhizal root colonization also provides a bioprotective effect against a broad range of soil-borne fungal pathogens (Dehne, 1982; Singh et al., 2000; St-Arnaud and Vujanovic, 2006). Take-all disease, which is caused by *Gaeumannomyces graminis* var. *tritici* (Ggt), is the most important root disease of cereal plants such as wheat, barley and rye (Asher and Shipton, 1981; Hornby et al., 1998; Cook, 2003), however, only few reports are available on a bioprotective effect of AMF on Ggt. It has been

reported that root colonization by AMF locally reduces lesions caused by Ggt in wheat roots (Graham and Menge, 1982) and enhances the shoot and root growth of plants colonized by Ggt compared to non-mycorrhizal Ggt-colonized wheat plants (Graham and Menge, 1982; Ksiezniak et al., 2001).

Some data on a local bioprotective effect of mycorrhizal root colonization against soil-borne fungal pathogens have been reported (Dehne, 1982; Singh et al., 2000; St-Arnaud and Vujanovic, 2006), however, only scarce data are available on a systemic bioprotective effect of mycorrhizal root colonization. In two reports with *Phytophthora parasitica* and tomato the development of a systemic resistance through root colonization by AMF has been suggested (Cordier et al., 1998; Pozo et al., 2002). Although a systemic resistance towards Ggt has been reported with sterile red fungus, a basidiomycete, which is known to suppress infection of wheat roots by Ggt (Aberra et al., 1998), no data are available yet on a systemic bioprotective effect of mycorrhizal colonization against take-all disease caused by Ggt.

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It is generally accepted that a well established AM symbiosis is a pre-requisite for a mycorrhizal-induced increase in resistance (Caron et al., 1986; Cordier et al., 1998; Slezack et al., 2000), and this also has been shown with Ggt. Whereas low degrees of AM colonization showed no effect on the disease development of Ggt, high degrees of AM colonization clearly reduced disease severity (Graham and Menge, 1982), however, no data are available yet whether a systemic bioprotective effect against soil-borne fungal pathogens also depends on the degree of AM root colonization.

Systemic acquired resistance (SAR) is a general response of plants to infectious agents (Ryals et al., 1996) which requires activation by previous pathogen infection and the accumulation of the signal molecule salicylic acid (SA) prior to its onset (Métraux et al., 1990). Thus, the accumulation of SA has been proposed as a marker for plant resistance (Klessig and Malamy, 1994), linking enhanced SA concentrations in plants to a reduced susceptibility of these plants to pathogens.

In the present work, we studied a possible systemic bioprotective effect of mycorrhizal colonization on the root pathogen Ggt. Moreover, we tested whether a systemic bioprotective effect of AM on Ggt depends on the degree of root colonization by the AMF *G. mosseae* and whether this systemic bioprotective effect can be linked with the accumulation of SA.

2. Materials and methods

2.1. Biological material and growth conditions

The pathogen *Gaeumannomyces graminis* var. *tritici* obtained from Centraalbureau voor Schimmelcultures/The Netherlands (CBS 541.86) and the AMF *Glomus mosseae* (Nicolson & Gerdemann) Gerd. & Trappe (BEG 12; La Banque Européenne des Glomales; International Institute of Biotechnology; Kent; GB) were used.

Barley (*Hordeum vulgare* L. cv. Xanadu) seeds were surface-sterilized by soaking in 0.75% sodium hypochlorite for 5 min, rinsed with tap water and germinated in vermiculite. After 5 d the seedlings were transferred to a steam-sterilized (20 min, 121 °C) mixture of silicate sand, expanded clay and soil (1:1:1; by vol.) into compartment boxes. Experiments were performed in a growth chamber (day/night cycle: 16 h; 23 °C/8 h; 19 °C; rel. humidity 50%). Plants were watered three times a week.

2.2. Compartment boxes and split-root system

Five days old barley plants were transferred into split-root systems (five plants per compartment). The split-root experimental system consists of two compartment units, each containing a half of the barley root system (for details see Vierheilig et al. (2000a) and Fig. 1). The two compartments are separated on the side joining each other by an impermeable PVC screen in order to prevent any

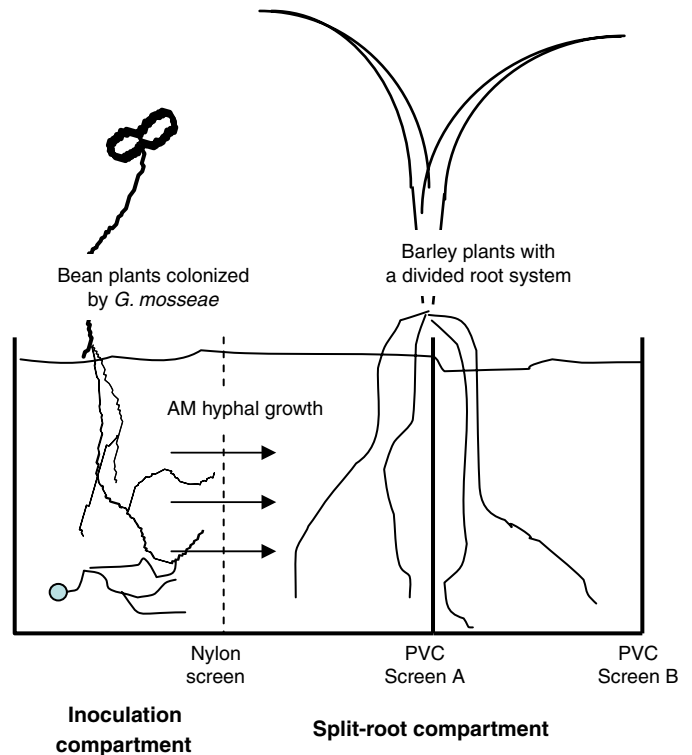


Fig. 1. Compartment system for inoculation with AMF and Ggt. The AMF inoculation compartment was joined with one side of split-root compartment simultaneously with Ggt-inoculation, 7 d before Ggt-inoculation or 14 d before Ggt-inoculation. This resulted in a different degree of AM root colonization on this side of the split-root system. Seven days before Ggt-inoculation the whole compartment system was inclined (45° angle) in such a way that roots grew onto PVC screen B. Thereafter the screen B was removed, the Ggt-inoculum applied and screen B replaced.

flow of molecules or root or hyphal growth from one to the other side. Thus one side of the split-root system can be inoculated with AMF without inoculating the other side.

2.3. AM inoculation

For AMF inoculation the outer side of each split-root compartment is equipped with a nylon screen (30 μm mesh), which can be penetrated by hyphae but not by roots. To inoculate half the split barley root system with *G. mosseae*, the outer side of a split-root compartment was joined with 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 (Fig. 1). The inoculum compartment contained beans (*Phaseolus vulgaris* L. cv. Sun Gold) colonized by *G. mosseae*.

Two days after transferring plants into the split-root compartments in the first treatment five plants were inoculated on one side of the split-root system with the AMF (14 d prior to Ggt inoculation; AM root colonization at time of Ggt inoculation $19 \pm 3\%$ (mean \pm SE)). In the second treatment 7 d after planting barley into the split-

root compartments another five plants were inoculated (7 d prior to Ggt inoculation; AM root colonization at time of Ggt inoculation $6 \pm 0.5\%$).

In a third treatment (16 d after transferring plants into the compartments) split-root systems of plant were simultaneously inoculated on one side with AMF and on the other side with Ggt (AM root colonization at time of Ggt inoculation 0%).

In order to obtain a homogenous Ggt infection 7 d 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 d the PVC screen could be removed and the inoculum (8 g barley seeds with growing Ggt/per plant) could be applied directly onto the roots. After application of the inoculum the PVC screen was replaced.

AM root colonization was determined at the time of Ggt inoculation (values see above) in five plants inoculated the same way as explained above.

In the control treatment plants were inoculated on one side of the split-root system with Ggt without AM colonization on the other side of the split-root system.

2.4. Ggt inoculum

G. graminis (Ggt) was cultured at 25°C for 7 d in the dark on Potato Dextrose agar (39 g^{-1})(Fluka). From the edge of the colony agar discs (with a diameter of 8 mm) with vigorously growing mycelium were removed and transferred to Erlenmeyer flasks containing 20 g of autoclaved barley seeds (1 h at 121°C). The inoculated barley seeds were incubated with light for 3–4 weeks at 25°C . From this inoculum 8 g per split-root/plant were applied (application method described by Mathre, 1992).

2.5. Effect of mycorrhizal colonization on SA accumulation at time of inoculation with Ggt

In a separate experiment plants were grown in order to determine the SA accumulation in split-root systems of barley plants with different degrees of mycorrhizal colonization on one side of the split-root system.

Barley plants were transferred into split-root systems (five plants per compartment) (Fig. 1). Two days after transferring plants into the split-root compartments in the first treatment five plants were inoculated on one side of the split-root system with the AMF (14 d prior to harvest; AM root colonization at time of harvest $21 \pm 8\%$). In the second treatment 7 d after planting barley into the split-root compartments another five plants were inoculated (7 d prior to harvest; AM root colonization at time of harvest $12 \pm 0.2\%$). Non-inoculated control plants were grown similarly and harvested after 16 d.

2.6. Determination of mycorrhizal colonization and severity caused by Ggt

At the time of harvest (14 d after Ggt inoculation; total plant age 5 weeks) roots were carefully rinsed with water. Shoot and root (from each side of the split-root system) fresh weight (FW) was determined and in fresh roots the percentage of Ggt infection was determined by scoring visibly lesioned roots. Lesioned roots could be easily identified due to alterations of root colour. Whitish roots were scored as non-infected, whereas yellow to dark brown root sections were scored as infected by Ggt (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 (5 min boiling in 10% KOH) and stained according to the method of Vierheilig et al. (1998) by boiling in a 5% ink (Shaeffer; black)/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).

Experiments were repeated twice with five replicates per treatment.

2.7. Extraction and quantification of SA

For the determination of the SA concentration after rinsing with water roots were frozen (-80°C) and thereafter lyophilised. Free SA and its glucoside were extracted and quantified as described by Malamy et al. (1992). One gram of frozen root tissue was ground in 3 ml of 90% methanol and centrifuged at $6000g$ for 15 min. The pellet was re-extracted with 3 ml of 100% methanol and centrifuged. Methanol extracts were combined, centrifuged for 10 min and dried at 40°C under vacuum. For each sample, the dried methanol extract was resuspended in 5 ml of water at 80°C , and the solution was divided into two equal portions. For the determination of the SA glucoside (SAG) to one portion an equal volume of 0.2 M acetate buffer (pH 4.5) containing 2 U/ml β -glucosidase (Sigma) for digestion was added, while to the other portion (free SA analysis) only buffer was added. Both portions were incubated at 37°C overnight. Thereafter, samples were acidified with HCl to pH 1 and SA was extracted with two volumes of cyclopentane/ethyl acetate/isopropanol (50:50:1) solvent. The lower aqueous phase was re-extracted with other two volumes of the same solvent and the total organic extract was dried under nitrogen, resuspended in $50\ \mu\text{l}$ of 100% methanol and analysed by HPLC.

Ten microliter of methanolic extract was injected into a C8 column (Varian) and phenolic compounds were separated with 30% (vol/vol) methanol in 1% acetic acid with a flow rate of 1.0 ml/min. at 40°C . SA was identified with a fluorescence detector set (Varian) at 310 and 405 nm

(excitation and emission, respectively) and quantified with a Data Module, using authentic standards. As has been described previously (Yalpani et al., 1993) the recovery of SA from tobacco using this methodology ranged between 30% and 50%.

2.8. Statistical analysis

Analysis of variance was done after a variance check by Levene's test. Statistics were carried out using Statgraphics Plus, Version 5.0.

3. Results

3.1. Systemic effect of mycorrhizal colonization

In the split-root system simultaneous inoculation with Ggt and the AMF on both sides and intermediate AMF root colonization on one side (degree AM root colonization at time of Ggt inoculation $6 \pm 0.5\%$ (mean \pm SE) and at the end of the experiment $36 \pm 11\%$) showed no effect on the percentage of roots lesion by Ggt, whereas with a high AMF root colonization on one side (degree AM root colonization at time of Ggt inoculation $19 \pm 3\%$ and at the end of the experiment $56 \pm 9\%$) the percentage of lesioned roots on the other side of the split-root system significantly dropped (Fig. 2).

None of the treatments did affect the shoot FW except in the treatment with the highest degree of AM root colonization the shoot FW was enhanced (1.10 ± 0.16 g FW) compared to the non-inoculated control plants (0.82 ± 0.10 g FW, Fig. 3).

Ggt inoculation resulted in all treatments (with AM inoculation and without AM inoculation) in a similarly reduced root FW on the Ggt inoculated side of the split-root system, however, Ggt inoculation on one side did not affect the root FW on the other side (Fig. 4).

3.2. Effect of mycorrhizal colonization on local and systemic SA accumulation

A significant locally enhanced accumulation of free SA could be detected in roots with an AM root colonization of around 20%, whereas an AM root colonization of around 10% showed no effect on SA accumulation (Fig. 5). No systemic effect of AM root colonization on SA accumulation could be observed in any of the treatments. The accumulation pattern of SAG was similar to the free SA (data not shown).

4. Discussion

In a study with wheat, Ggt and AMF, Graham and Menge (1982) showed that mycorrhizal plants clearly reduced the negative effect of take-all disease on plant growth. We found a slight positive effect of mycorrhizal colonization on shoot FW of Ggt inoculated plants only when plants were highly mycorrhizal. This contrasting observation might be due to the different experimental set-up. While Graham and Menge (1982) tested plant growth over 9 weeks, we harvested our plants after 5 weeks, only 14d after inoculation with Ggt. Thus, possibly Ggt infection still could not result in an effect on plant growth.

However, in our study mycorrhizal colonization showed a clear systemic effect on the number of lesioned roots.

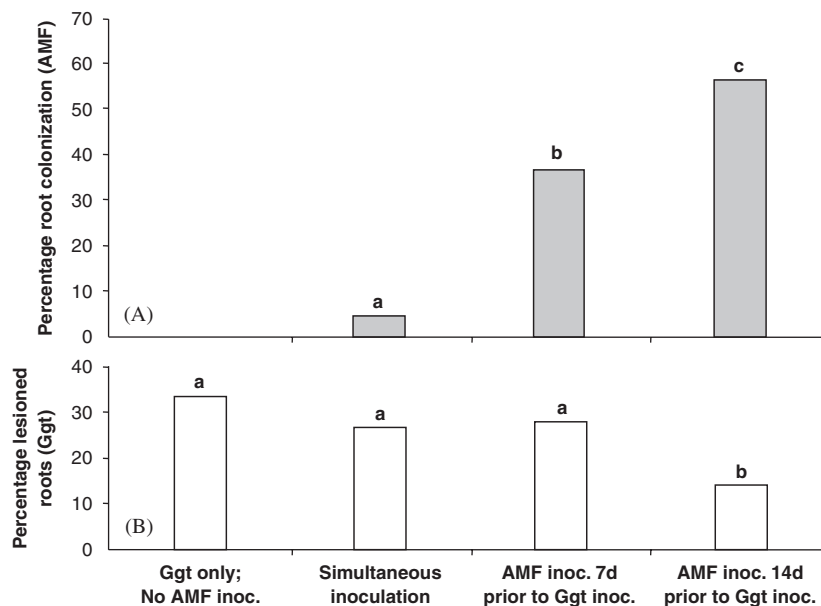


Fig. 2. Split root systems of barley with one side of the split-root system sequentially inoculated with the AMF *G. mosseae*, and the other side inoculated with *G. graminis* (Ggt). Degree of AM root colonization ($P = 0.000$) and (B) severity of take-all disease ($P = 0.0403$) at the end of the experiment. Columns followed by the same letter are not significantly different according to Fisher's least significant difference test.

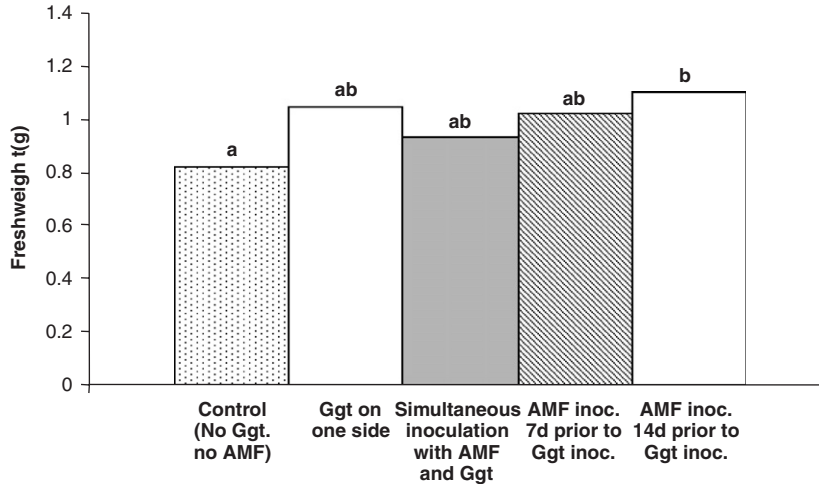


Fig. 3. Shoot freshweight of split-root systems of barley plants with one side sequentially inoculated with the AMF *G. mosseae* and the other side inoculated with *G. graminis* (Ggt). Columns followed by the same letter are not significantly different according to Fisher's least significant difference test ($P = 0.0456$).

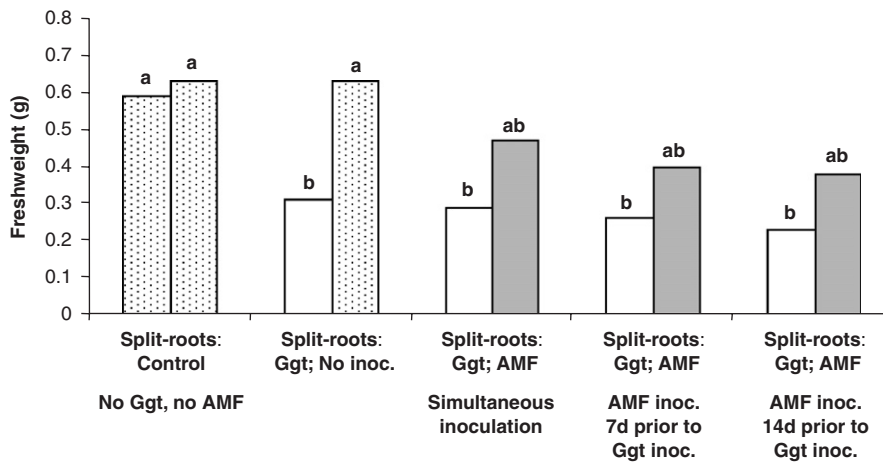


Fig. 4. Root freshweight of split-root systems of barley plants with one side sequentially inoculated with the AMF *G. mosseae* and the other side inoculated with *G. graminis* (Ggt). Columns followed by the same letter are not significantly different according to Fisher's least significant difference test ($P = 0.0412$).

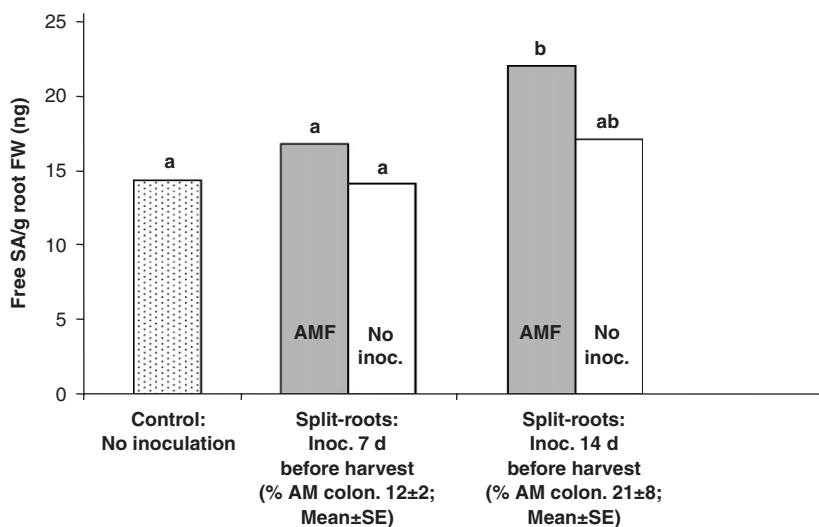


Fig. 5. Concentration of free SA in split-root systems of barley with the first side inoculated (AMF) with *G. mosseae* at different times and the second side without inoculation (No inoc.). Columns followed by the same letter are not significantly different according to Fisher's least significant difference test ($P = 0.0229$).

First data on a systemic bioprotective effect of mycorrhizal colonization have been provided with *P. parasitica* and tomato (Cordier et al., 1998; Pozo et al., 2002). We show that a systemic bioprotection through mycorrhizal colonization is a more general response and occurs also with barley and Ggt, although this effect is clearly linked with the degree of AM root colonization. A high degree of AM root colonization in a split-root system systemically reduced the number of lesioned roots, whereas in roots with low and intermediate degrees of AM colonization the number of lesioned roots was similar to the non-mycorrhizal control.

These findings corroborate studies on non-systemic bioprotection obtained with *P. parasitica* or *Fusarium oxysporum* and tomato (Caron et al., 1986; Cordier et al., 1998), *Aphanomyces euteiches* and pea (Slezack et al., 2000), and Ggt in wheat (Graham and Menge, 1982) suggesting that a high degree of AM root colonization is essential for a mycorrhizal-induced increase in resistance. In the present study we could show for the first time that a high degree of AM root colonization is not only essential for local bioprotection as shown before (Caron et al., 1986; Cordier et al., 1998; Graham and Menge, 1982; Slezack et al., 2000), but also for systemic bioprotection.

This systemic bioprotective effect of mycorrhizal colonization depending on the degree of root colonization by an AMF reminds of a recent observation in mycorrhizal autoregulation. Mycorrhizal autoregulation means that once one side of a split-root system is mycorrhizal, mycorrhizal colonization on the second side of the split-root system is systemically suppressed (Vierheilig et al., 2000a, b; Catford et al., 2003, 2006; Meixner et al., 2005). Recently it has been suggested that mycorrhizal autoregulation might be regulated similarly as systemic bioprotection towards soilborne fungal pathogens observed in mycorrhizal plants. It seems more than plausible that a mycorrhizal plant activates only one mechanism to repulse further root colonization by fungi, not discriminating between AMF and soilborne pathogenic fungi (Vierheilig and Piché, 2002; Vierheilig, 2004a). This hypothesis is further strengthened by our data and recent data by Vierheilig (2004b). We found that Ggt severity was only systemically reduced at higher degrees of AM pre-colonization (>50%) on one side of a split-root system. Vierheilig (2004b) found an identical pattern in barley when looking at mycorrhizal autoregulation. Higher degrees of AM root colonization (>50%) on one side systemically suppressed mycorrhizal colonization at the other of a split-root system.

In several reports P-concentrations have been reported to affect take-all disease. P-deficiency always increased the incidence and/or severity of take-all disease (Mattingly et al., 1980; Reis et al., 1981; Graham and Menge, 1982; Brennan 1988). As mycorrhizal colonization improves the P-status of plants (Smith and Read, 1997) the enhanced P-concentration in mycorrhizal plants might reduce the effects of Ggt. In the present study we did not determine

P-concentrations in the barley plants, however, recently in a similar experimental set-up with barley we determined the P-concentrations in split-root systems with one side colonized by the AMF *G. mosseae* and the other side non-mycorrhizal (Vierheilig et al., 2000b). Whereas P-concentrations on the mycorrhizal side of the split-root system were clearly increased, P-concentrations on the non-mycorrhizal side were similar to the non-mycorrhizal control plants. These data exclude a direct effect of the P-concentrations in the root on Ggt, however, as P can also induce systemic effects (Walters et al., 2005), P cannot be excluded to be involved in the observed systemic bioprotective effect against Ggt.

Some reports on the concentrations of SA in barley leaves have been published before (Vallelian-Bindschedler et al., 1998; Hueckelhoven et al., 1999; Chaman et al., 2003), however, no data were available yet on SA concentrations in roots of barley. We could show that the concentrations of free SA detected in the roots of barley are similar to those reported for barley leaves (Vallelian-Bindschedler et al., 1998) and are in the same range as recently reported for tobacco roots (Medina et al., 2003).

There is some information available on the involvement of SA in the regulation of root colonization by AMF (Blilou et al., 1999; Ludwig-Müller et al., 2002; Medina et al., 2003), indicating that enhanced SA concentrations in plants are linked with a reduced susceptibility of plants to AM colonization. However, nearly no data are available on SA and the susceptibility of mycorrhizal plants to pathogen infection. Working with a NahG tobacco mutant that is unable to accumulate SA, Shaul et al. (1999) showed that alterations of the infection of leaves by *Botrytis cinerea* in mycorrhizal tobacco plants are SA-independent.

Virtually no data are available on Ggt and SA except one report by Seah et al. (1996). In this work SA when exogenously applied to wheat was not effective in the reduction of the susceptibility of the seedlings to Ggt suggesting that SA is not involved in a plant resistance response to Ggt. When we determined the SA concentration at the time before inoculation with Ggt, with degrees of AM colonization around 10% and 20%, we observed only with the higher degree of mycorrhizal colonization a SA accumulation in roots colonized by the AMF, however, in none of the treatments SA was systemically accumulated. These results could point towards an involvement of SA in local resistance against Ggt, but this seems in contrast with the observations by Seah et al. (1996) suggesting no role of SA in resistance against Ggt. However, it should be kept in mind that SA could act differently when applied exogenously or when accumulated in the plant tissue. Further studies are necessary to clearly elucidate the role of SA in the host-Ggt interaction.

To summarize, we could demonstrate that in barley AM root colonization results in a systemic bioprotective effect against Ggt. In analogy to a local bioprotective effect this systemic bioprotection depends on a high degree of AM root colonization. Studies are underway to

further elucidate a putative role of SA in mycorrhizal bioprotection.

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