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ORIGINAL PAPER

S. Scheffknecht · R. Mammerler · S. Steinkellner · H. Vierheilig

Root exudates of mycorrhizal tomato plants exhibit a different effect on microconidia germination of *Fusarium oxysporum* f. sp. *lycopersici* than root exudates from non-mycorrhizal tomato plants

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Abstract The effect of root exudates from mycorrhizal and non-mycorrhizal tomato plants on microconidia germination of the tomato pathogen *Fusarium oxysporum* f. sp. *lycopersici* was tested. Microconidia germination was enhanced in the presence of root exudates from mycorrhizal tomato plants. The more tomato plants were colonized by the arbuscular mycorrhizal fungus *Glomus mosseae*, the more microconidia germination was increased, indicating that alterations of the exudation pattern depended on the degree of root AM colonization. Moreover, alterations of the exudation pattern of mycorrhizal plants are not only local, but also systemic. Testing the exudates from plants with a high and a low P level revealed that the alterations of the root exudates from mycorrhizal plants, resulting in a changed effect on microconidia germination, are not due to an improved P status of mycorrhizal plants.

Keywords Arbuscular mycorrhiza · Glomales · Fusarium oxysporum · Microconidia · Tomato · Root exudates

Introduction

Arbuscular mycorrhizal fungi (AMF) are symbiotic soil fungi that colonize roots of about 80% of vascular plants. The mycorrhizal symbiosis enhances the growth and survival of numerous plant species (Smith and Read 1997). The establishment of the highly complex mycorrhizal association requires a continuous exchange of signals between the host roots and AMF, which affects the whole metabolism of the host (Smith and Read 1997).

It is reasonable to speculate that changes in the metabolism of the mycorrhizal host plant also result in a changed root exudation, which, in consequence, exhibits a different bioactive effect on organisms around the root (Vierheilig and Piché 2002; Vierheilig 2004a). In in vitro conditions, the first evidence of an altered root exudation of mycorrhizal plants has been provided with exudates from cucumber plants. Root exudates of mycorrhizal cucumber plants showed a reduced stimulatory effect on AM hyphal growth and an inhibitory effect on root colonization by AMF (Pinior et al. 1999; Vierheilig et al. 2003). Moreover, a changed exudation pattern of mycorrhizal plants has been suggested to be at least partially involved in the altered susceptibility of mycorrhizal plants towards soil-borne microorganisms (Vierheilig and Piché 2002; Vierheilig 2004a) such as fungi, bacteria, and nematodes. In in vitro studies, root exudates from mycorrhizal strawberry plants reduced the sporulation of *Phytophthora fragariae* (Norman and Hooker 2000) and root exudates from mycorrhizal potato plants increased hatching of nematodes (Ryan and Jones 2004).

Recently, it has been shown that root exudates collected from non-mycorrhizal tomato roots exhibit a higher attracting effect on zoospores of *Phytophthora parasitica* than root exudates from mycorrhizal tomato roots (Lioussanne et al. 2003). An inverse effect was observed with the chemotactic response of plant-growth-promoting bacteria. Root exudates from mycorrhizal tomato plants showed a higher attractional effect on plant-growth-promoting bacteria, such as *Azobacter chroococum* and *Pseudomonas fluorescens*, compared to root exudates from non-mycorrhizal tomato plants (Sood 2003).

Although there are some data on the effect of root exudates from mycorrhizal plants on symbiotic and pathogenic soil microorganisms, nothing is known yet on the effect of root exudates from mycorrhizal plants on the microconidia germination of the tomato pathogen *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*).

S. Scheffknecht · R. Mammerler · S. Steinkellner (✉) · H. Vierheilig
Institut für Pflanzenschutz, Department für Angewandte Pflanzenwissenschaften, und Pflanzenbiotechnologie,
Universität für Bodenkultur Wien,
Peter-Jordan-Str. 82,
A-1190 Wien, Austria
e-mail: siegrid.steinkellner@boku.ac.at
Tel.: +43-1-476543352
Fax: +43-1-476543359

Fol is an important pathogen in tomato cultures. Extensive studies have been performed, in detail, on the infection process of *Fusarium oxysporum* (Lagopodi et al. 2002; Rodríguez-Gálvez and Mendgen 1995); however, still few information is available about the pre-infection stages (Deacon 1996) of *Fol*, such as triggering of spore germination. To study the early signalling steps of the host–*Fol* interaction, Steinkellner et al. (2005) recently developed an in vitro bioassay that looked at the microconidia germination of *Fol* in the presence of root exudates.

To elucidate changes of the root exudation in mycorrhizal plants affecting microconidia germination of *Fol* in the present study, we: 1) compared the effect of root exudates from mycorrhizal and non-mycorrhizal tomato plants, 2) tested how the degree of mycorrhization affects the root exudation, 3) verified if these changes in the exudation pattern are systemic or local, and 4) verified if these changes depend on the P status of the tomato plant.

Materials and methods

Plant material and growth conditions

Seeds of *Lycopersicon esculentum* Mill. cv. Moneymaker, were surface-sterilized in 50% commercial bleach for 5 min, rinsed several times in sterile distilled water, and germinated in autoclaved (20 min; 121°C) perlite. After germination, plantlets were transferred in the compartment system developed by Wyss et al. (1991) in a steam-sterilized (20 min; 121°C) mixture of silicate sand, expanded clay, and soil (1:1:1; by volume).

Plants were grown in a growth chamber (day/night cycle: 16 h; 23°C/8 h; 19°C; relative humidity 50%). The experiments were performed with *Glomus mosseae* (BEG 12; International Bank of Glomeromycota; International Institute of Biotechnology, Kent, Great Britain).

Root exudates from mycorrhizal and non-mycorrhizal tomato plants

Fourteen days after seeding, five plantlets were transferred into the compartment system and inoculated with *G. mosseae* (for details, see Wyss et al. 1991). Twenty one days after inoculation, plants were harvested, root exudates collected, and the degree of root colonization determined (55%±7) as described below.

Root exudates from tomato plants with a different degree of root colonization

Twelve days after seeding, plantlets were transferred into the compartment system. To obtain a different degree of root colonization in plants of the same age, plants had to be inoculated sequentially.

In the first treatment, five plants were inoculated immediately after the transfer, resulting 25 days after inoculation in a degree of root colonization of 29%±5. In the second treatment, five plants were inoculated 13 days after transferring plants into the compartment, resulting 12 days after inoculation in a degree of root colonization of 13%±3. In the third treatment, five plants were inoculated 19 days after transferring plants into the compartment, resulting 7 days after inoculation in a degree of root colonization of 4%±2. At the end of the experiment, root exudates were collected, and the degree of root colonization was determined.

Root exudates from tomato plants with a different P status

Fourteen days after seeding, plantlets were transferred into the compartment system and inoculated with *G. mosseae* (for details, see Wyss et al. 1991). One patch of plants received once-a-week 5 ml of a P solution (0.125 g KH_2PO_4/100 ml H_2O dest.). Twenty-one days after inoculation, plants were harvested, root exudates collected, the degree of root colonization determined (44%±5), and the P content in leaves was determined with the ammonium–vanadat–molybdenum method (Gericke and Kurmies 1952).

Root exudates from tomato plants in a split-root system

Twelve days after seeding, the main root of the tomato seedlings was cut. Thereafter, plants were grown for another 21 days in the mixture of silicate sand, expanded clay, and soil (for details, see above), then five plants were transferred into a split-root compartment system (for details, see Vierheilig et al. 2003) in the same substrate, and one side of the split-root system was immediately inoculated with *G. mosseae* (see Vierheilig et al. 2003).

Sixteen days after inoculation, plants were harvested, root exudates of the mycorrhizal and the non-mycorrhizal side collected, and the degree of root colonization was determined on the mycorrhizal side (44%±5) and the non-mycorrhizal side (0%).

Extraction and treatment of root exudates

At the end of the experiments, intact mycorrhizal plants were harvested, roots were rinsed with tap water and roots were placed in beakers (24 h in the growth chamber; for details on the growth chamber, see above) filled with distilled water to obtain root exudates. Root exudates of non-mycorrhizal plants, grown in the same way but non-inoculated, were also collected.

For collection of exudates from the split-root system, roots from the mycorrhizal side and the non-mycorrhizal side of the split-root system of the tomato plants were placed in two separate beakers (24 h in the growth chamber; for more details, see above) filled with distilled water to obtain root exudates. Subsequently, the fresh

weight of the root was determined. The exudate obtained was adjusted with sterilized water to 20 ml per gram root fresh weight and the pH was measured. The pH ranged from 6.35 to 7.15. In a pre-experiment, we tested the effect of the pH on microconidia germination. Czapek Dox solutions were adjusted to pH 5.4, 5.8, 6.2, 6.6, 7.0, 7.4, and 7.8. By statistical analysis, no difference of microconidia germination (*P* value=0.2681) could be observed.

The exudates were passed through 0.22 μm sterilfilters (MicronSep, Cellulosic, Roth, Germany) and stored at −20°C until use.

Fungal cultures

F. oxysporum f. sp. *lycopersici* was grown on Czapek Dox (CzD) agar at 24°C in darkness. The *F. oxysporum* f. sp. *lycopersici* isolate *Fol* 007 was kindly provided by B.J. Cornelissen, Institute for Molecular Cell Biology, Amsterdam. Under sterile conditions, fungal cultures were flooded with sterilized water and the suspension was filtered through three layers of filter paper (Vliesscheiben für Kannenfilter, Laporte Ges.m.b.H., Wels, Austria). The spore-suspension was concentrated by centrifugation at 3,000×*g* for 10 min and adjusted to 1.0×10^7 microconidia/ml water using a haemocytometer.

Germination experiments

The germination assay was performed in sterile culture plates (24 wells) (Greiner bio-one, No. 662160, Frickenhausen, Germany). Aliquots of 500 μl of root exudate were mixed with 100 μl of spore suspension and incubated a 24°C in the dark while shaking at 200 rpm. Microconidia germination was determined microscopically after 24 h by counting 200 spores. The experiments were performed in triplicates and replicated three times. Sterilized water and CzD solution were included in the germination experiments as a control.

Estimation of root colonization

To visualize the AMF colonization, roots were cleared by boiling 4 min in 10% KOH, rinsed three times with tap water, and stained according to the method of Vierheilig et al. (1998) by boiling for 4 min in a 5% ink (Shaeffer; black)/household vinegar (=5% acetic acid) solution. After staining, the percentage of root colonization was determined according to the method of Newman (1966).

Statistical analysis

An analysis of variance was done after a variance check by Levene's test. Mean values were compared using Fisher's least significant difference. These analyses were performed using Statgraphics Plus 5.0.

Results

Root exudates from mycorrhizal and non-mycorrhizal tomato plants

The effect of root exudates from mycorrhizal and non-mycorrhizal tomato plants on microconidia germination was tested (Fig. 1). Microconidia germination was highest in the control treatment with the CzD medium and lowest in the control treatment with water. Treatment with root exudates from non-mycorrhizal tomato plants resulted in an enhanced spore germination, compared to the water control. With root exudates from mycorrhizal tomato plants present, spore germination was more than doubled, compared to spore germination in the presence of root exudates from non-mycorrhizal tomato plants.

Root exudates from tomato plants with a different degree of root colonization

The effect of root exudates from tomato plants with a different degree of root colonization on microconidia germination was tested (Fig. 2). Colonization levels by the AMF G. mosseae in tomato roots ranged from 4%±2 and 13%±3 to 29%±5.

Root exudates from tomato plants with a low degree of root colonization (4% and 13%) enhanced microconidia germination slightly compared to root exudates from non-mycorrhizal plants. With root exudates from tomato plants with a higher degree of root colonization (29%) microconidia germination was even more increased.

Root exudates from tomato plants with a different P status

Plants were inoculated with the AMF, non-inoculated, or non-inoculated with a P treatment. The P content was determined in the leaves, and the effect of root exudates from non-mycorrhizal (−P and +P) and mycorrhizal tomato plants on microconidia germination was tested.

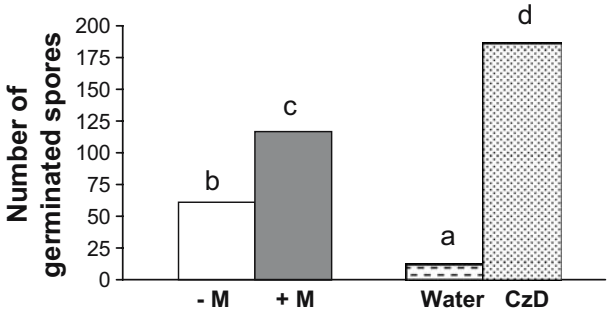

Fig. 1 Effect of water, Czapek Dox (CzD) solution, root exudates from mycorrhizal (+M) (55%±7) and non-mycorrhizal (−M) tomato plants on microconidia germination of *Fusarium oxysporum* f. sp. *lycopersici*. Columns followed by differing letters are significantly different, according to Fisher's LSD test (*P*=0.0000)

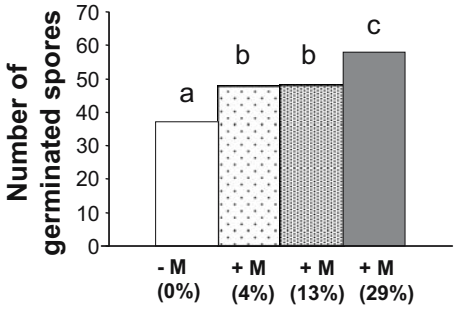

Fig. 2 Effect of root exudates from tomato plants with a different degree of AM root colonization (in brackets % of root colonization) on microconidia germination of *Fusarium oxysporum* f. sp. *lycopersici*. Columns followed by the same letter are not significantly different, according to Fisher's LSD test (P=0.0006)

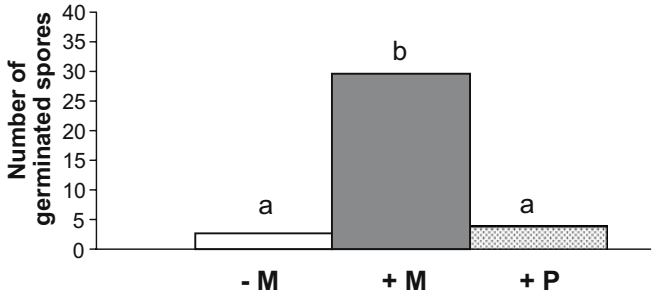

Fig. 4 Effect of root exudates from of mycorrhizal (+M) (44%±5) and non-mycorrhizal (−M) tomato plants or from non-mycorrhizal tomato plants supplied with a P solution (+P) on microconidia germination of *Fusarium oxysporum* f. sp. *lycopersici*. Columns followed by the same letter are not significantly different, according to Fisher's LSD test (P=0.0002)

The P content in the leaves was significantly enhanced in the tomato plants with a P application, whereas, in the mycorrhizal plants, the P content was not enhanced, compared to the non-mycorrhizal −P plants (Fig. 3). No difference of the effect of root exudates from −P and +P plants on *Fol* microconidia germination could be observed; however, in the presence of root exudates from mycorrhizal tomato plants *Fol* microconidia germination was significantly enhanced (Fig. 4).

Root exudates from tomato plants with a split-root system

The effect of root exudates from tomato plants with a split-root system (with one side mycorrhizal and the other side non-mycorrhizal) on microconidia germination was tested (Fig. 5). In the presence of root exudates from the mycorrhizal side of the split-root system, microconidia germination was more than doubled, compared to microconidia germination in the presence of root exudates from non-mycorrhizal control plants. In the presence of root exudates from the non-mycorrhizal side of the split-root system of a mycorrhizal plant, the germination rate was even slightly reduced, compared to germination in the presence of root exudates from non-mycorrhizal control plants.

Discussion

Changes of the root exudation pattern through mycorrhization expressed as a different effect of root exudates on soil microorganisms have been reported in a number of studies and have been suggested to be at least partially involved in the altered susceptibility of mycorrhizal plants towards soil-borne microorganisms (Jones et al. 2004; Vierheilig and Piché 2002; Vierheilig 2004a).

In our study, root exudates of tomato plants seemed to be altered through mycorrhization. Microconidia germination in the presence of root exudates from mycorrhizal tomatoes was clearly increased, compared to germination in the presence of root exudates from non-mycorrhizal plants, indicating that the changes in the root exudates through mycorrhization were favorable to *Fol* microconidia germination.

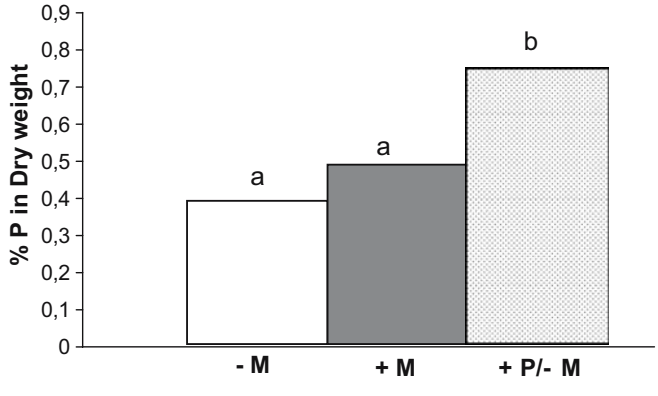

Fig. 3 P content in leaves of mycorrhizal (+M) and non-mycorrhizal (−M) tomato plants and in non-mycorrhizal tomato plants supplied with a P solution (+P/−M). Columns followed by the same letter are not significantly different, according to Fisher's LSD test (P=0.0004)

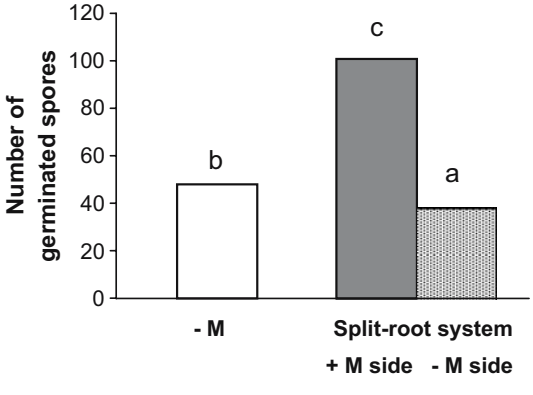

Fig. 5 Effect of root exudates from non-mycorrhizal tomato plants (−M) (44%±5) and tomato plants with a split-root system with one side mycorrhizal (+M side) and the other side non-mycorrhizal (−M side) on microconidia germination of *Fusarium oxysporum* f. sp. *lycopersici*. Columns followed by differing letters are significantly different, according to Fisher's LSD test (P=0.0000)

These data seem in contrast with reports on the susceptibility of mycorrhizal tomato plants to *F. oxysporum*. In several studies a bioprotectional effect of AM to *F. oxysporum* f. sp. *lycopersici* (Akköprü and Demir 2005) and *F. oxysporum* f. sp. *radicis-lycopersici* (Caron et al. 1985, 1986a) has been reported. However, the microconidia germination-enhancing effect of root exudates from mycorrhizal tomato plants we observed in our study does not necessarily mean that other stages of the infection process are affected similarly. Root exudates from mycorrhizal tomato plants could affect hyphal growth, the formation of penetration structures or the final penetration, differently than microconidia germination, thus, resulting in a reduced susceptibility of the mycorrhizal plant, or, in the tomato–*Fol* interaction, root exudates have a minor role as plant signals, and, thus, changes in the root exudation pattern do not affect the infection by *Fol*.

The altered effect of root exudates from mycorrhizal tomato plants on *Fol* microconidia germination we observed could be due to: 1) the presence of new compounds in root exudates stimulatory for microconidia germination, 2) enhanced levels of compounds stimulatory for microconidia germination, or 3) reduced levels of inhibitory compounds in addition to an overall stimulatory effect of the root exudates.

In several studies, it has been reported that root exudates of high P plants show a different effect on soil-borne fungi, than root exudates from low P plants (Elias and Safir 1987; Tawaraya et al. 1996, 1998). The main nutritional effect of mycorrhization is the improved P-nutritional status of the host plants (Smith and Read 1997); thus, the root exudation pattern of mycorrhizal plants could be altered due to the improved P uptake. In our study, testing root exudates of non-mycorrhizal tomato plants with a high P status and with a low P status revealed that they exhibited a similar effect on microconidia germination, whereas, germination was increased with root exudates from mycorrhizal tomato plants. This clearly proves that the observed different effect of mycorrhizal and non-mycorrhizal root exudates on microconidia germination is not due to an improved P status of the mycorrhizal plants.

An effect of the degree of root colonization by AMF on further root colonization by pathogenic and symbiotic fungi has been reported in several studies (Caron et al. 1986b; Cordier et al. 1998; Vierheilig 2004b); however, no clear data on the mechanisms involved are available yet. Interestingly, in our study, the effect of root exudates from mycorrhizal tomato plants also depended on the degree of mycorrhizal colonization. In the presence of root exudates from tomato plants with a high degree of root colonization, microconidia germination was higher than in the presence of root exudates from tomato plants with a low degree of root colonization. Our results indicate that the different infection by soil-borne fungi of mycorrhizal plants with different degrees of root AM colonization could be at least partially linked to a root exudation, which is regulated by the degree of mycorrhization of the host plant.

In mycorrhizal plants, alterations of the exudation pattern seem not to be limited to mycorrhizal roots, but, through a plant-mediated mechanism, occur also in non-mycorrhizal roots of a mycorrhizal root system (Jones et al. 2004; Vierheilig 2004a). Our study confirmed systemic alterations of the exudation pattern of mycorrhizal plants. As mentioned above, exudates from mycorrhizal roots clearly increased the microconidia germination of *Fol*. To our surprise, we observed a contrary effect in the presence of root exudates from non-mycorrhizal roots of a mycorrhizal tomato plant. In this case, microconidia germination was significantly lower than with the exudates from the non-mycorrhizal control plants.

This is in contrast with other reports on root exudates. When comparing the bacterial community structure in the rhizosphere of mycorrhizal and non-mycorrhizal maize plants, Marschner and Baumann (2003) found changes around the roots of mycorrhizal plants. Interestingly, they observed similar changes of the bacterial community structure on the non-mycorrhizal side of split-root system with one side mycorrhizal and the other side non-mycorrhizal, showing clearly that the alterations of the root exudation pattern were not only local but systemic.

Most recently, systemic alterations of the root exudation pattern through mycorrhization have also been reported with cucumber root exudates. Exudates of a split-root system of cucumber, with one side mycorrhizal and the other side non-mycorrhizal, exhibited a similar inhibitory effect on root colonization by AMF, showing that changes on the exudation pattern in mycorrhizal cucumber plants are systemic, which means not limited to mycorrhizal root (Vierheilig et al. 2003).

To summarize, our study clearly shows that the altered exudation pattern of mycorrhizal plants expressed as a different bioactive effect on soil-microorganisms is also affecting the tomato pathogen *Fol*, thus, indicating that alterations of the root exudation pattern through mycorrhization are an important general biological phenomena. Moreover, alterations of the exudation pattern of mycorrhizal plants are not only local, but systemic. Testing the exudates from plants with a high and a low P level, clearly revealed that the alterations of the root exudates from mycorrhizal plants, resulting in a changed effect on microconidia germination, are not due to an improved P status of mycorrhizal plants.

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References

Akköprü A, Demir S (2005) Biological control of Fusarium wilt in tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* by AMF *Glomus intraradices* and some rhizobacteria. J Phytopathol 153:544–550

Caron M, Fortin JA, Richard C (1985) Effect of *Glomus intraradices* on infection by *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomatoes over a 12-week period. Can J Bot 64:552–556

Caron M, Fortin JA, Richard C (1986a) Effect of inoculation sequence on the interaction between *Glomus intraradices* and *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomatoes. Can J Plant Pathol 8:12–16

Caron M, Fortin JA, Richard C (1986b) Effect of phosphorus concentration and *Glomus intraradices* on Fusarium crown and root rot of tomatoes. Phytopathology 76:942–946

Cordier C, Pozo MJ, Barea JM, Gianinazzi S, Gianinazzi-Pearson V (1998) Cell defense responses associated with localized and systemic resistance to *Phytophthora parasitica* induced in tomato by an arbuscular mycorrhizal fungus. Mol Plant Microb Interact I 11:1017–1028

Deacon JW (1996) Ecological implications of recognition events in the pre-infection stages of root pathogens. New Phytol 133:135–145

Elias KS, Safir GR (1987) Hyphal elongation of *Glomus fasciculatus* in response to root exudates. Appl Environ Microbiol 53:1928–1933

Gericke S, Kurmies B (1952) Die kolorimetrische Phosphorsäure-bestimmung mit Ammonium – Vanadat – Molybdat und ihre Anwendung in der Pflanzenanalyse. Z Pflanzenernähr, Düngung, Bodenkunde 59:235–247

Jones DL, Hodge A, Kuzyakov Y (2004) Plant and mycorrhizal regulation of rhizodeposition. New Phytol 163:459–480

Lagopodi AL, Ram AFJ, Lamers GEM, Punt PJ, Van den Hondel CAMJ, Lugtenberg BJJ, Bloemberg GV (2002) Novel aspects of tomato root colonization and infection by *Fusarium oxysporum* f. sp. *radicis-lycopersici* revealed by confocal laser scanning microscopic analysis using the green flourescent protein as a marker. Mol Plant Microb Interact I 15:172–179

Lioussanne L, Jolicoeur M, St. Arnaud M (2003) Effects of the alteration of tomato root exudation by *Glomus intraradices* colonization on *Phytophthora parasitica* var. Nicotianae zoospores. Abstract No. 253, Abstract Book ICOM 4; Montreal, Canada, p 291

Marschner P, Baumann K (2003) Changes in bacterial community structure induced by mycorrhizal colonisation in split-root maize. Plant Soil 251:279–289

Newman EI (1966) A method of estimating the total length of root in a sample. J Appl Ecol 3:139–145

Norman JR, Hooker JE (2000) Sporulation of *Phytophthora fragariae* shows greater stimulation by exudates of non-mycorrhizal than by mycorrhizal strawberry roots. Mycol Res 104:1069–1073

Pinior A, Wyss U, Piché Y, Vierheilig H (1999) Plants colonized by AM fungi regulate further root colonization by AM fungi through altered root exudation. Can J Bot 77:891–897

Rodríguez-Gálvez E, Mendgen K (1995) The infection process of *Fusarium oxysporum* in cotton root tips. Protoplasma 189:61–72

Ryan A, Jones P (2004) The effect of mycorrhization of potato roots on the hatching chemicals active towards the potato cyst nematodes, *Globodera pallida* and *G. rostochiensis*. Nematology 6:335–342

Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic, London

Sood SG (2003) Chemotactic response of plant-growth-promoting bacteria towards roots of vesicular–arbuscular mycorrhizal tomato plants. FEMS Microbiol Ecol 45:219–227

Steinkellner S, Mammerler R, Vierheilig H (2005) Microconidia germination of the tomato pathogen *Fusarium oxysporum* in the presence of root exudates. J Plant Interact 1:23–30

Tawaraya K, Watanabe S, Yoshida E, Wagatsuma T (1996) Effect of onion (*Allium cepa*) root exudates on the hyphal growth of *Gigaspora margarita*. Mycorrhiza 6:57–59

Tawaraya K, Hashimoto K, Wagatsuma T (1998) Effect of root exudate fractions from P-deficient and P-sufficient onion plants on root colonisation by the arbuscular mycorrhizal fungus *Gigaspora margarita*. Mycorrhiza 8:67–70

Vierheilig H (2004a) Regulatory mechanisms during the plant-arbuscular mycorrhizal fungus interaction. Can J Bot 82:1166–1176

Vierheilig H (2004b) Further root colonization by arbuscular mycorrhizal fungi in already mycorrhizal plants is suppressed after a critical level of root colonization. J Plant Physiol 161:339–341

Vierheilig H, Piché Y (2002) Signalling in arbuscular mycorrhiza: facts and hypotheses. In: Buslig B, Manthey J (eds) Flavonoids in cell function. Kluwer Academic/Plenum, New York, pp 23–39

Vierheilig H, Coughlan AP, Wyss U, Piché Y (1998) Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Appl Environ Microbiol 64:5004–5007

Vierheilig H, Lerat S, Piché Y (2003) Systemic inhibition of arbuscular mycorrhiza development by root exudates of cucumber plants colonized by *Glomus mosseae*. Mycorrhiza 13:167–170

Wyss P, Boller T, Wiemken A (1991) Phytoalexin response is elicited by a pathogen (*Rhizoctonia solani*) but not by a mycorrhizal fungus (*Glomus mosseae*) in bean roots. Experientia 47:395–399