Mycorrhiza (2006) 16: 413–419
DOI 10.1007/s00572-006-0051-z

ORIGINAL PAPER

Maendy Fritz · Iver Jakobsen · Michael Foged Lyngkjær · Hans Thordal-Christensen · Jörn Pons-Kühnemann

Arbuscular mycorrhiza reduces susceptibility of tomato to *Alternaria solani*

Received: 17 October 2005 / Accepted: 7 March 2006 / Published online: 14 April 2006

Abstract Mycorrhiza frequently leads to the control of root pathogens, but appears to have the opposite effect on leaf pathogens. In this study, we studied mycorrhizal effects on the development of early blight in tomato (*Solanum lycopersicum*) caused by the necrotrophic fungus *Alternaria solani*. *Alternaria*-induced necrosis and chlorosis of all leaves were studied in mycorrhizal and non-mycorrhizal plants over time course and at different soil P levels. Mycorrhizal tomato plants had significantly less *A. solani* symptoms than non-mycorrhizal plants, but neither plant growth nor phosphate uptake was enhanced by mycorrhizas. An increased P supply had no effect on disease severity in non-mycorrhizal plants, but led to a higher disease severity in mycorrhizal plants. This was parallel to a P-supply-induced reduction in mycorrhiza formation. The protective effect of mycorrhizas towards development of *A. solani* has some parallels to induced systemic resistance, mediated by rhizobacteria: both biocontrol agents are root-associated organisms and both are effective against necrotrophic pathogens. The possible mechanisms involved are discussed.

Keywords *Alternaria solani* · Arbuscular mycorrhizas · *Glomus intraradices* · Induced systemic resistance · *Solanum lycopersicum*

M. Fritz (✉) · J. Pons-Kühnemann
Biometry and Population Genetics
Justus-Liebig-Universität Gießen,
IFZ, Heinrich-Buff-Ring 26-32,
35392 Gießen, Germany
e-mail: Maendy.Fritz@agrar.uni-giessen.de
Tel.: +49-641-9937547
Fax: +49-641-9937549

I. Jakobsen · M. F. Lyngkjær
Biosystems Department, Risø National Laboratory,
Roskilde, Denmark

H. Thordal-Christensen
Department of Agricultural Sciences,
The Royal Veterinary and Agricultural University,
Copenhagen, Denmark

Introduction

Early blight of tomato, caused by the fungus *Alternaria solani*, is one of the common foliar diseases of tomatoes. This disease can occur over a wide range of climatic conditions, but is most severe in areas with high relative humidity caused by heavy dew or rainfall combined with high averaged temperatures. Tomato crops are damaged by damping-off of seedlings, collar rot, destruction of foliage as well as direct destruction of fruits by fruit rot and sunscald of fruits on defoliated plants (Rotem 1994). *Alternaria* spp. fungi produce many non-host-specific as well as host-specific toxins (Thomma 2003), which kill the host cells before or at the time of invasion and the fungus subsequently lives as saprophyte on the decaying tissue. In contrast to such necrotrophic lifestyle, biotrophic fungi feed on living host cells and are very host specific pathogens.

Strategies for the control of *A. solani* include the activation of various forms of induced resistance. Systemic acquired resistance (SAR) is activated after infection by a necrotising pathogen or other biotic and abiotic stresses, rendering distant, uninfected plant parts resistant towards a broad spectrum of pathogens (Durrant and Dong 2004). Certain strains of plant growth promoting rhizobacteria are able to induce systemic resistance, which extends to the above-ground plant parts and is phenotypically similar to SAR. This second type of induced disease resistance is commonly referred to as rhizobacteria-mediated, induced systemic resistance (ISR), as reviewed by Van Loon et al. (1998). Based on work with gene-knockout mutants in *Arabidopsis*, SAR and ISR are proposed to confer resistance to pathogens according to their lifestyles, so that SAR primarily functions against biotrophic pathogens and ISR against necrotrophic pathogens (Thomma et al. 1998, 2001). Parallel to the induction of resistance, significant changes in the plant occur: inoculation with root-promoting *Pseudomonas* results in a significant increase in phenylpropanoid content in sprouts (Leinhos and Bergmann 1995) and root exudates (Azcón-Aguilar and Barea 1995). ISR can be induced not only by the rhizobacteria

themselves, but also by bacteria-synthesised macromolecules (Romeiro et al. 2005).

Root colonisation by arbuscular mycorrhizal fungi (AMF) has been frequently reported to reduce root infection by various soil-borne pathogens (Azcón-Aguilar and Barea 1996; Smith and Read 1997). The mechanisms involved in this biocontrol are not clear, but localised and systemic induced resistance (Cordier et al. 1998) as well as increase in plant P status in response to mycorrhiza formation (Graham 2000) appear to be involved. In contrast, mycorrhiza is generally assumed to increase susceptibility to leaf pathogens such as fungi, viruses and aphids (Dehne 1982; Lindermann 1994).

The objective of this work was to investigate effects of mycorrhiza on foliar susceptibility to *A. solani* in tomato. Disease symptoms were studied over time in mycorrhizal and non-mycorrhizal plants, and the possible role of mycorrhiza-induced changes in plant P status was investigated by supplying different amounts of P to both non-mycorrhizal and mycorrhizal plants.

Materials and methods

Experimental design

Two experiments were carried out: experiment 1 had four treatments: 0 P, 25 P, 75 P, and 0 P + AMF (numbers refer to milligrams of P added kg^{-1} soil). Experiment 2 had eight treatments resulting from combining four P levels with the presence or absence of mycorrhizas: 0 P, 0 P + AMF, 3 P, 3 P + AMF, 6 P, 6 P + AMF, 24 P, and 24 P + AMF. The experiments were conducted in a completely randomised design and repeated once. In general, all treatments had four replicates as indicated by figures and tables. For experiment 2, seven plants per treatment were grown and three of these were harvested before inoculation with *A. solani*, reducing the replications to three for these data. Later data of this experiment were obtained from the four remaining plants.

Biological materials

Lycopersicon esculentum cv. Frembgens Rheinlands Ruhm was grown in both experiments. Seeds were surface-sterilised (95% EtOH, soaked in 4% NaOCl (v:v) for 10 min and a final rinse in distilled H_2O) and pre-germinated on wet filter paper for 2 days. Mycorrhizal plants were established in both experiments by inoculation with *Glomus intraradices* (BEG 87), which had been propagated on *Trifolium subterraneum*. The dry inoculum consisted of soil, hyphae, spores and colonised root pieces. The *A. solani* isolate Greece-1 was kindly provided by Simon Pérez Martinez, CENSA, Cuba, and was cultured on potato dextrose agar at 25°C. To harvest the spores, 10-day-old cultures were brushed gently to loosen the spores and then rinsed with a 0.01% Tween 20 solution. The resulting spore suspension was filtered through a fine cloth, quantified using a haemocytometer and adjusted to 10^4 spores ml^{-1}.

Experimental setup

Plants were grown in square pots (8 cm side length) lined with plastic bags and filled with 400 g growth medium, which was a 1:1 (w:w) mixture of quarts sand and irradiated soil (10 kGy, 10 MeV electron beam). The growth medium, hereafter referred to as soil, had nutrients uniformly incorporated at the following concentrations (milligram per kilogram dry soil): K_2SO_4 75.0; $CaCl_2 \cdot 2H_2O$ 75.0; $CuSO_4 \cdot 5H_2O$ 2.1; $ZnSO_4 \cdot 7H_2O$ 5.4; $MnSO_4 \cdot H_2O$ 10.5; $CoSO_4 \cdot 7H_2O$ 0.39; $MgSO_4 \cdot 7H_2O$ 45.0; $Na_2MoO_4 \cdot 2H_2O$ 0.18; and NH_4NO_3 30.0 (Viereck et al. 2004). The soil had a bicarbonate-extractable P content of 9.8 μg P g^{-1} (Olsen et al. 1954). The various P levels in the two experiments were established by thorough mixing of KH_2PO_4 into the soil. Mycorrhizal treatments (+ AMF) had 32 g of the soil replaced by *G. intraradices* inoculum. The soil was moistened to 60% of water holding capacity, and pots were left for 1 week for incubation of AMF inoculum. Three pre-germinated seeds were planted in each pot and were thinned to one plant per pot after establishment. The soil surface was covered with plastic beads. Plants were grown in a climate chamber with a 16: 8 h light:dark cycle with 24–26:18–20°C temperatures, respectively, and watered daily to 60% water holding capacity. Plants were inoculated 35 days after sowing by spraying with spore suspension until run-off occurred. To ensure high relative humidity that is needed during spore germination, the plants were placed in tight closing clear plastic containers for 24 to 48 h.

Monitoring of disease severity, harvest and analyses

Early blight disease severity was assessed daily and every second day in experiments 1 and 2, respectively, by assigning a grade in the range 1–12 reflecting the percentage of leaf area with necrotic spots and chlorosis, respectively, of all unfolded leaves. Grade 12 was assigned to dead and shed leaves. For each time point, arithmetic means for individual plants were calculated. Tomato shoots were harvested 5 and 8 days after infection (dai) with *A. solani* in experiments 1 and 2, respectively, and weighed after drying at 70°C for 2 days. Ground shoot material was digested in a 4:1 mixture (v:v) of nitric and perchloric acids. Total P content was measured by the molybdate blue method (Murphy and Riley 1962) on a Technicon Auto-analyser II (Technicon Autoanalysers, Analytical Instruments Recycle, Golden, CO, USA). Root systems were washed and root samples of mycorrhizal tomatoes were cleared with 10% KOH and stained with 0.05% trypan blue in lactoglycerol (Phillips and Hayman 1970) with the omission of phenol from the solutions and HCl from the rinse. Percentage of AMF colonisation was determined

using a gridline intersection method (Giovanetti and Mosse 1980).

Statistical analysis

Data for dry weight, phosphate content and mycorrhizal colonisation were analysed using GLM and Tukey's test. Necrosis and chlorosis data, which were surveyed over several days in all experiments, were analysed with a linear mixed model for repeated measurements, using the auto-regressive covariance structure which fitted best with respect to the experimental structure and minimised values for Akaike's Information Criterion (AIC) and other information criteria. This statistical approach compares the treatments during the whole period of data collection and not only at certain time points by estimation of new means which are representing the whole time course and are therefore slightly different to the measured values. Degrees of freedom were estimated according to Satterthwaite's formula (Satterthwaite 1946 after Hocking 1996). Levels of P addition and inoculation with *G. intraradices* were set as fixed factors, whereas the intervals between the assessments were used as covariates. Treatments were compared using LSD test with Bonferroni correction. All calculations were performed using SPSS (SPSS for Windows, Rel. 12.0.1, 2003. Chicago, SPSS).

Results

In experiment 1, the *A. solani* development was determined (1) on non-mycorrhizal tomato plants with increasing P supplies and (2) on mycorrhizal and non-mycorrhizal tomato plants given no additional P. Disease severity in terms of necrosis (P=0.005) and chlorosis (P=0.000) was significantly reduced by mycorrhiza. Whereas, the effect of increased P supply to non-mycorrhizal plants was not significant (P=0.208 and P=0.089 for necrosis and chlorosis, respectively) (Fig. 1; Table 1). Necrosis and chlorosis increased significantly (both P=0.000) over time in all treatments. Interactions could not be tested due to the incomplete experimental design. The increased degree of necrosis in response to P addition was not significant. For the range of concentrations used in this study, phosphate nutrition had no influence on severity of early blight. The more pronounced effect on disease development arose from the addition of *G. intraradices* to the 0 P treatment (0 P + AMF) such that mycorrhizal tomato plants had significantly less *Alternaria* symptoms in terms of necrosis and chlorosis than non-mycorrhizal plants (Fig. 1, Table 1).

To test whether the reduced *Alternaria* symptoms in the mycorrhizal plants in the first experiment were caused by an improved phosphate level, a second experiment was set up where mycorrhiza was combined with all phosphate levels in the range between 0 and 24 mg kg^{-1}, resulting in a complete factorial design. Phosphate concentrations in shoots were analysed to study whether disease severity was somehow correlated to any change in leaf P status caused by AMF inoculation or P supply.

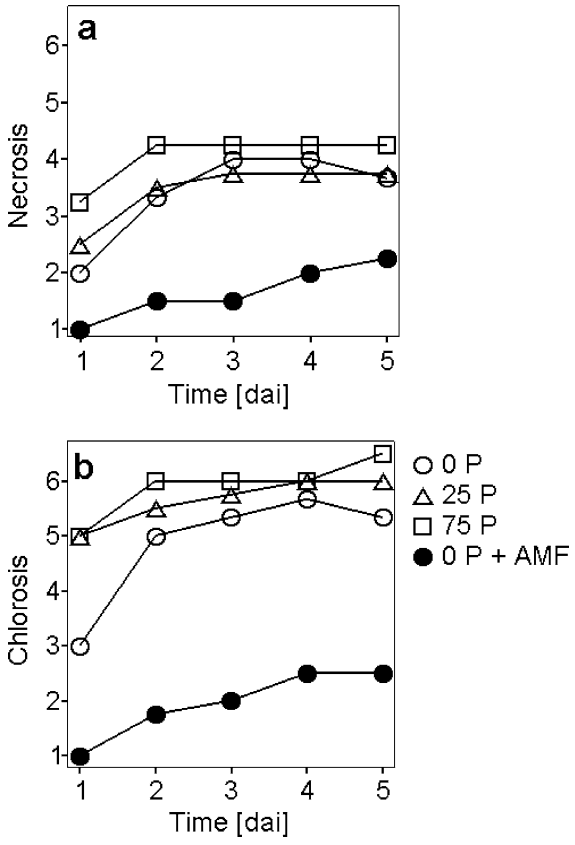

Fig. 1 Necrosis (**a**) and chlorosis (**b**) caused by *Alternaria solani* infection monitored over 5 days after inoculation (dai) of tomato leaves. 0, 25 and 75 P represent non-mycorrhizal plants with different levels of phosphate (P) supply (0, 25, 75 mg kg^{-1} soil) and 0 P + AMF represents mycorrhizal plants. *Data points* show arithmetic means with n=4

A two-way statistical analysis showed that disease severity was significantly influenced by the addition of AMF (P=0.000 for both necrosis and chlorosis). Mycorrhizal plants had significantly less disease symptoms (Fig. 2a and Table 2). Necrotic symptoms did not differ at various levels of P supply (P=0.253) but chlorosis of

Table 1 Necrosis and chlorosis ranking of mycorrhizal (+ AMF) and non-mycorrhizal tomato plants supplied (25 and 75 mg kg^{-1} soil) or not (0 P) with phosphate and estimated over a time period of 5 days after infection with *Alternaria solani*

Treatment	Necrosis	Chlorosis
0 P	3.16 b	4.55 b
25 P	3.31 b	5.58 b
75 P	3.92 b	5.83 b
0 P + AMF	1.64 a	1.86 a

Estimated means were calculated in a mixed model with repeated measures data. The standard errors of necrosis means are SE=0.34 for 0 P and SE=0.29 for all other treatments. For chlorosis data, the standard errors are SE=0.41 and SE=0.36. Degrees of freedom are df=11.89 for necrosis data and df=11.81 for chlorosis. Means followed by *equal letters* are not significantly different

leaves was significantly influenced by the amount of additional P (P=0.014). Also, there were significant interactions between mycorrhiza and the level of P supply, with P=0.019 and P=0.002 for necrosis and chlorosis, respectively. These interactions were very obvious as an increase in P supply caused more early blight symptoms in mycorrhizal plants. At the 24 P supply level, mycorrhizal and non-mycorrhizal tomatoes did not differ significantly (Table 2). Seven days after inoculation with *A. solani*, the remaining non-mycorrhizal tomato plants had lost between 13 and 22% of their dry matter, as many chlorotic leaves were shed. Mycorrhizal tomato plants suffered much less, resulting in higher dry weights after infection with early blight, though this difference was only significant at the 0 P level due to high variability of the data (Fig. 2b).

The dry weight of mycorrhizal and non-mycorrhizal tomatoes was quantified before and after inoculation with *A. solani* (Fig. 2c,d). Before inoculation with *A. solani*, dry matter of plants was slightly higher when more phosphate

Table 2 Necrosis and chlorosis ranking of mycorrhizal (+ AMF) and non-mycorrhizal tomato plants supplied (3, 6 and 24 mg kg^{-1} soil) or not (0 P) with phosphate and estimated over a time period of third to seventh day after infection with *Alternaria solani*

Treatment	Necrosis	Chlorosis
0 P	4.42 d	4.50 b
0 P + AMF	1.35 ab	1.63 a
3 P	3.31 cd	3.75 b
3 P + AMF	1.15 a	1.50 a
6 P	3.48 cd	4.38 b
6 P + AMF	1.29 ab	2.00 a
24 P	3.08 bcd	3.88 b
24 P + AMF	2.50 abc	3.75 b

Estimated means were calculated in a mixed model with repeated measures data. The standard error of necrosis means is SE=0.37 and the degrees of freedom are df=25.79 for all treatments, whereas the values are SE=0.33 and df=24.00 for chlorosis data. Means followed by *equal letters* are not significantly different

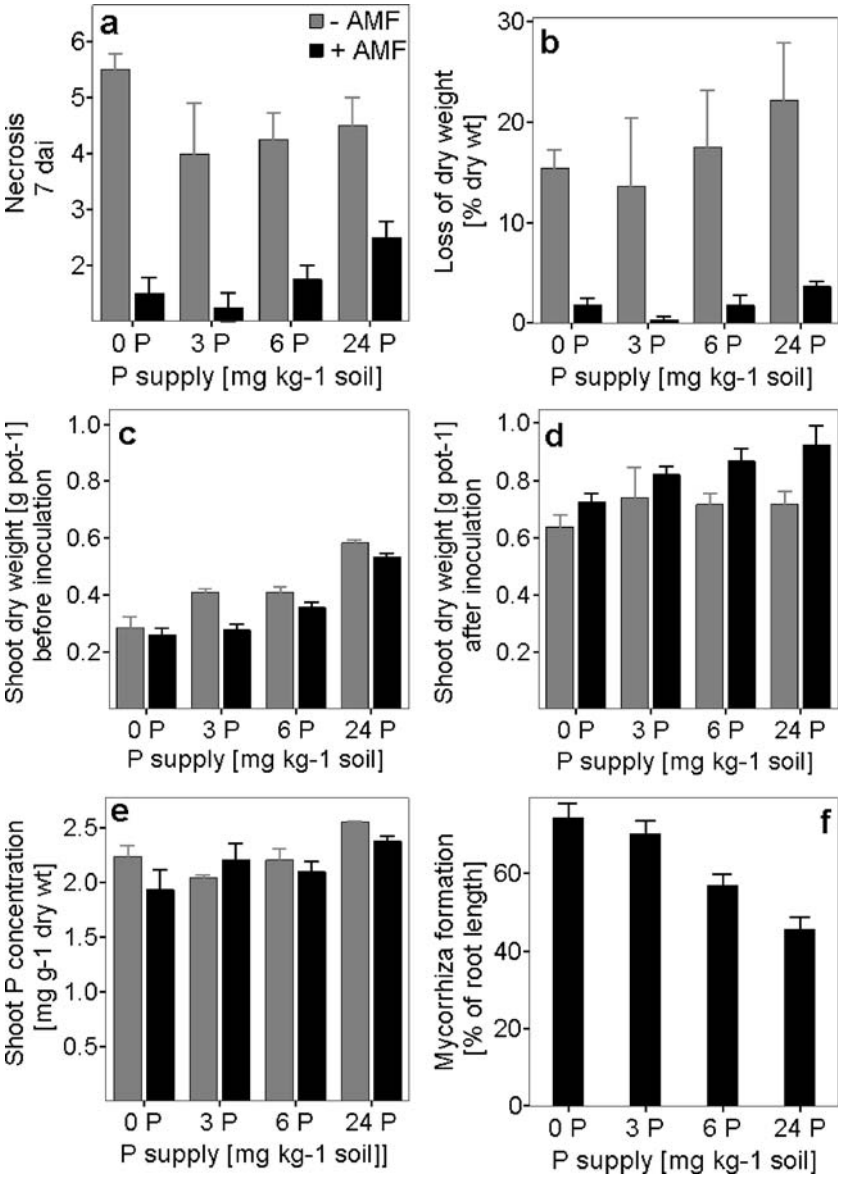

Fig. 2 Leaf necrosis (**a**), necrosis-induced dry weight loss (**b**), shoot dry weight (**c**, **d**), shoot P concentration (**e**) and mycorrhiza levels (**f**) in mycorrhizal (+ AMF) and non-mycorrhizal (− AMF) tomato plants grown at four levels of phosphate (P) supply. Shoot dry weights in (**c**) were recorded just before inoculation with *Alternaria solani* on different plants whilst all other data were recorded 7 days after inoculation (dai). *Columns* represent arithmetic means and *bars* are standard errors [n=3 for (**c**) and otherwise 4]

was added, and non-mycorrhizal tomatoes were generally larger than mycorrhizal ones even if these differences were rarely significant (see Fig. 2c). The P content in milligram per gram dry weight in shoots before infection was nearly at the same level for all treatments (Fig. 2e); only the treatments 0 P + AMF and 3 P contained significantly lower phosphate than plants of treatment 24 P. As expected, mycorrhizal colonisation was significantly reduced when more phosphate was added (Fig. 2f), and this reduction could explain the described interactions.

Discussion

The presence of mycorrhiza in tomato roots led to significantly lower infection levels of *A. solani* than observed in non-mycorrhizal plants in two separate experiments. Bioprotection of AM-colonised plants against soil-borne pests like nematodes and various root diseases is commonly observed (Cordier et al. 1996; Boedker et al. 1998). Some studies have shown mycorrhizal protection of tomato plants against, for example, the root pathogens *Phytophthora parasitica*, *Erwinia carotovora* and *Pseudomonas syringae* (García-Garrido and Ocampo 1988, 1989; Cordier et al. 1996, 1998). In contrast, susceptibility to leaf pathogens is often higher in mycorrhizal than in non-mycorrhizal plants (Lindermann 1994). Dehne (1982) suggested that the systemic influence of AMF may be attributed to enhanced nutrition, plant growth and physiological activity of mycorrhizal plants, and therefore, with increased levels of assimilates, such plants can serve as improved nutrient sources for plant parasitic organisms. Shaul et al. (1999) provided an alternative mechanism by explaining the increased disease severity by the suppression of the plant defence response by AMF shortly after the early events of root colonisation. However, mycorrhiza may induce qualitative or quantitative changes in plant performance that could compensate higher disease susceptibility. Hence, although mycorrhiza formation in barley resulted in increased leaf infection by *Blumeria graminis* f. sp. *hordei*, mycorrhizal plants suffered less than non-mycorrhizal plants in terms of reductions in grain number, ear yield and thousand-grain weight (Gernns et al. 2001).

Alternaria fungi are saprophytes and facultative parasites with necrotrophic nature that may explain why they require a weakened, stressed or senescent host plant for infection (Rotem 1994). Rotem et al. (1990) showed for *Alternaria macrospora* that the disease severity of cotton plants grown in cool regime, causing delayed aging, was lower than for those grown under hot conditions (causing accelerated aging). This demonstrates that physiological rather than chronological age governs the age-conditioned susceptibility. AM fungi constitute a sink for the carbon resources of the host and mycorrhizal plants require improved production of assimilates (Smith and Read 1997; Gernns et al. 2001). As carbon costs can be as high as 20% (Jakobsen and Rosendahl 1990; Douds et al. 2000; Graham 2000), competition for carbon between hosts and AM fungi can be strong. This competition can result in a growth reduction of mycorrhizal plants compared to non-mycorrhizal ones, especially under light deficiency or other photosynthesis-limiting condition. An age delaying effect of AMF on senescence has been observed in leaves (Gernns et al. 2001) and roots (Gavito et al. 2001; Lingua et al. 2002) of crop plants. In our experiments, the tomato plants colonised by an AMF did not show a positive growth effect due to mycorrhization and even a slight growth depression was observed in terms of fresh and dry matter (before inoculation with early blight), which is similar to that observed in previous studies in our laboratory (Burleigh et al. 2002; Smith et al. 2004). Competition for carbon compounds could have been a cause for decreased pathogen development in mycorrhizal plants as the growth of both symbiotic and pathogenic organisms depends on host photosynthates (Azcón-Aguilar et al. 2002). The nutritional status can be an important factor influencing the disease susceptibility of plants. Our experiments demonstrated that additional P did not increase early blight symptoms, but only reduced formation of mycorrhiza. The phosphate concentration of mycorrhizal tomato was not increased by *G. intraradices*. Thus, the increase in resistance in the treatments with AMF cannot be explained by a better P supply. Other nutrients such as nitrogen, potassium or zinc were not measured during the studies, as only surplus supplies of N are known to reduce early blight symptoms (Vintal et al. 1999).

An early mycorrhizal inoculation, previous to pathogen attack, has been shown to be a successful practice to increase disease tolerance/resistance in economically important crop species mainly for those involved in horticultural and fruit production systems (Lovato et al. 1996; Pinochet et al. 1998). In tomato plants infected with *P. parasitica*, only a well-established mycorrhizal colonization could protect plants (Cordier et al. 1996), and bioprotection by *Glomus mosseae* against *Aphanomyces euteiches* was shown to depend on a fully established symbiosis with presence of arbuscules (Slezak et al. 2000). In our first experiment, mycorrhizal colonisation was as high as 79% with no difference due to phosphate levels. The level of mycorrhizal development in our second study did depend significantly on P supply to the tomato plants and the decrease in root colonisation due to additional P could be an explanation for the lack of induced resistance in the treatment with high P supply.

Different organisms can stimulate plants and activate either pathogen-induced SAR or rhizobacteria-mediated ISR. Parallel to ISR, mycorrhizal fungi interact with the host plant's roots and influence the whole plant including the above-ground parts. ISR is effective against necrotrophic pathogens and it seems possible that similar mechanisms reduce susceptibility of mycorrhizal plants towards necrotrophic leaf pathogens like *A. solani*. Rhizobacteria, which were inevitably added together with the mycorrhiza inoculum in the + AMF pots, could probably be involved in the observed effects. Lindermann (2000) states that AMF-induced changes in the mycorrhizosphere could be involved in reduced disease of soil-borne pathogens, but they may also be involved in the ISR

effect. However, the reduced mycorrhiza formation in the plants of treatment 24 P + AMF of experiment 2 showed reduced mycorrhiza-induced resistance. This hints that mycorrhiza development itself is responsible for the observed reduction in foliar susceptibility to *A. solani*, as root protection against pathogens (Cordier et al. 1996, 1998) by AM fungi is necessary for protection.

We conclude that mycorrhization of tomato roots can induce reduced susceptibility to necrotrophic leaf pathogen *A. solani*. This effect is indirectly influenced by phosphate supply which reduces mycorrhizal development and leads to reduced resistance. The effect resembles the rhizobacteria-mediated, induced systemic resistance that is also effective against necrotrophic pathogens.

Acknowledgements The work described in this paper was carried out at Risø National Laboratory, Roskilde, Denmark. We thank Anne and Anette Olsen for the excellent technical assistance. The stay in Denmark was supported by the Marie Curie Fellowship Contract No. HPMT-CT-2000-0019.

References

Azcón-Aguilar C, Barea JM (1995) Saprophytic growth of arbuscular mycorrhizal fungi. In: Varma A, Hock B (eds) Mycorrhiza. Springer, Berlin Heidelberg New York, pp 391–467

Azcón-Aguilar C, Barea JM (1996) Arbuscular mycorrhizas and biological control of soil-borne plant pathogens—an overview of the mechanisms involved. Mycorrhiza 6:457–464

Azcón-Aguilar C, Jaizme-Vega MC, Calvet C (2002) The contribution of arbuscular mycorrhizal fungi to the control of soil-borne plant pathogens. In: Gianinazzi S, Schüepp H, Barea JM, Haselwandter K (eds) Mycorrhizal technology in agriculture: from genes to bioproducts. Birkhäuser Verlag, Boston, pp 187–198

Boedker L, Kjoeller R, Rosendahl S (1998) Effect of phosphate and the arbuscular mycorrhizal fungus *Glomus intraradices* on disease severity of root rot of peas (*Pisum sativum*) caused by *Aphanomyces euteiches*. Mycorrhiza 8:169–174

Burleigh SH, Cavagnaro TR, Jakobsen I (2002) Functional diversity of arbuscular mycorrhizas extends to the expression of plant genes involved in P nutrition. J Exp Bot 53:1593–1601

Cordier C, Gianinazzi S, Gianinazzi-Pearson V (1996) Colonisation patterns of root tissues by *Phytophthora nicotianae* var. *parasitica* related to reduced disease in mycorrhizal tomato. Plant Soil 185:223–232

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 Microbe Interact 1017–1028

Dehne HW (1982) Interaction between vesicular–arbuscular mycorrhizal fungi and plant pathogens. Phytopathology 72:1115–1119

Douds DD, Pfeffer PE, Shachar-Hill Y (2000) Carbon partitioning, cost and metabolism of arbuscular mycorrhizae in arbuscular mycorrhizas: physiology and function. In: Kapulnick Y, Douds DDJ (eds) Arbuscular mycorrhizas: molecular biology and physiology. Kluwer, Dordrecht, The Netherlands, pp 107–130

Durrant WE, Dong X (2004) Systemic acquired resistance. Annu Rev Phytopathol 42:185–209

García-Garrido JM, Ocampo JA (1988) Interaction between *Glomus mosseae* and *Erwinia carotovora* and its effects on the growth of tomato plants. New Phytol 110:551–555

García-Garrido JM, Ocampo JA (1989) Effect of VA mycorrhizal infection of tomato on damage caused by *Pseudomonas syringae*. Soil Biol Biochem 21:165–167

Gavito ME, Curtis PS, Jakobsen I (2001) Neither mycorrhizal inoculation nor atmospheric CO_2 concentration has strong effects on pea root production and root loss. New Phytol 149:283–290

Gernns H, von Alten H, Poehling H-M (2001) Arbuscular mycorrhiza increased the activity of a biotrophic leaf pathogen—is a compensation possible? Mycorrhiza 11:237–243

Giovanetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular–arbuscular mycorrhizal infection in roots. New Phytol 84:489–500

Graham JH (2000) Assessing costs of arbuscular mycorrhizal symbiosis in agroecosystems. In: Podila GK, Douds DDJ (eds) Current advances in mycorrhizae research. APS Press, St. Paul, Minnesota, pp 127–140

Hocking RR (1996) Methods and applications of linear models: regression and the analysis of variance. Wiley, New York, NY

Jakobsen I, Rosendahl L (1990) Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. New Phytol 115:77–83

Leinhos V, Bergmann H (1995) Effect of amino alcohol application, rhizobacteria and mycorrhiza inoculation on the growth, the content of protein and phenolics and the protein pattern of drought stress lettuce (*Lactuca sativa* L. cv. Amerikanischer brauner). J Applied Bot 69:153–156

Lindermann RG (1994) Role of VAM fungi in biocontrol. In: Pfleger FL, Linderman RG (eds) Mycorrhiza in plant health. The American Phytopathological Society Press, St. Paul, Minnesota, pp 1–25

Lindermann RG (2000) Effects of mycorrhizas on plant tolerance to diseases. In: Kapulnik Y, Douds DD (eds) Arbuscular mycorrhizas: Molecular Biology and Physiology. Kluwer, Dordrecht, The Netherlands, pp 345–365

Lingua G, D'Agostino G, Massa N, Antosiano M, Berta G (2002) Mycorrhiza-induced differential response to a yellows disease in tomato. Mycorrhiza 12:191–198

Lovato PE, Gianinazzi-Pearson V, Trouvelot A, Gianinazzi S (1996) The state of art of mycorrhizas and micropropagation. Adv Hortic Sci 46–52

Murphy J, Riley J (1962) A modified single solution method for determination of phosphate in natural water. Anal Chim Acta 27:495–501

Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circular No. 939, United States Department of Agriculture, Washington DC, USA

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. Trans British Mycol Soc 55:158–160

Pinochet J, Camprubí A, Calvet C, Fernández C, Rodríguez-Kábana R (1998) Inducing tolerance to the root-lesion nematode *Pratylenchus vulnus* by early mycorrhizal inoculation of micropropagated Myrobalan 29 C plum rootstock. J Am Soc Hortic Sci 123:342–347

Romeiro RS, Filho L, Vieira Junior JR, Silva HSA, Baracat-Pereira MC, Carvalho MG (2005) Macromolecules released by a plant growth-promoting rhizobacterium as elicitor of systemic resistance in tomato to bacterial and fungal pathogens. J Phytopathol 153:120–123

Rotem J (1994) The genus *Alternaria*: biology, epidemiology and pathogenicity. American Phytopathological Society Press, St. Paul, Minnesota

Rotem J, Drepper W, Bashi E, Kranz J (1990) The effect of age, nutrition and soil moisture on predisposition of cotton to infection by *Alternaria macrospora*. Phytopathol Mediterr 29:19–24

Satterthwaithe FE (1946) An approximate distribution of estimates of variance compounds. Biom Bull 2:110–114

Shaul O, Galili S, Volpin H, Ginzberg I, Elad Y, Chet I, Kapulnik Y (1999) Mycorrhiza-induced changes in disease severity and PR protein expression in tobacco leaves. Mol Plant Microbe Interact 12:1000–1007

Slezak S, Dumas-Gaudot E, Paynot M, Gianinazzi S (2000) Is a fully established arbuscular mycorrhizal symbiosis required for bioprotection of Pisum sativum root against Aphanomyces euteiches? Mol Plant Microbe Interact 13:238–241

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

Smith SE, Smith FA, Jakobsen I (2004) Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. New Phytol 162:511–524

Thomma BPHJ (2003) *Alternaria* spp.: from general saprophyte to specific parasite. Mol Plant Pathol 4:225–236

Thomma BPHJ, Eggermont K, Penninckx IAMA, Mauch-Mani B, Vogelsang R, Cammue BPA, Broekaert WF (1998) Separate jasmonate-dependent and salicylate-dependent defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. Proc Natl Acad Sci USA 95:15107–15111

Thomma BPHJ, Penninckx IAMA, Broekaert WF, Cammue BPA (2001) The complexity of disease signalling in *Arabidopsis*. Curr Opin Immunol 13:63–68

Van Loon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol 36:453–483

Viereck N, Hansen PE, Jakobsen I (2004) Phosphate pool dynamics in the arbuscular mycorrhizal fungus *Glomus intraradices* studied by in vivo ^{31}P NMR spectroscopy. New Phytol 162:783–794

Vintal H, Ben-Noon E, Shlevin E, Yermiyahu U, Shtienberg D, Dinoor D (1999) Influence of rate of soil fertilization on Alternaria leaf blight (*Alternaria dauci*) in carrots. Phytoparasitica 27:1–8