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Arbuscular mycorrhizal fungi reduce development of pea root-rot caused by *Aphanomyces euteiches* using oospores as pathogen inoculum

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Abstract

The effects of the arbuscular mycorrhizal (AM)-fungi *Glomus intraradices* and *Glomus claroideum* on pea root-rot development caused by the pathogen *Aphanomyces euteiches* were investigated in a greenhouse pot-experiment, over the course of three harvests, using oospores as pathogen inoculum. Signature whole cell fatty acids 16:1ω5c and 14:1ω9 were used to quantify AM-fungi and *A. euteiches*, respectively in both roots and soil. Disease incidence was reduced in AM plants, though this effect was more pronounced in plants with *G. intraradices* than plants with *G. claroideum*, and corresponded with a greater mycorrhiza development, both intra- and extra radical in plants with *G. intraradices* than with *G. claroideum*. At the final harvest, percentage of root length with oospores was similar in roots of mycorrhizal and non-mycorrhizal plants. Despite the fact that pea root-rot development was only slightly lower in mycorrhizal plants compared to that of non-mycorrhizal plants, in terms of shoot growth and disease severity, mycorrhizal plants suffered less. This suggests a possible mycorrhiza-induced tolerance against pea root-rot. Furthermore, the degree of tolerance induction differed between the two AM-fungi included in the present study.

Introduction

Aphanomyces euteiches causes pea (*Pisum sativum*) root-rot and is a serious root pathogen, world wide (Papavizas and Ayers, 1974; Kraft and Boge, 1994). Oospores constitute the primary inoculum source, which after germination produce zoospores constituting the actual root infective units. After root infection, the pathogen rapidly spreads in the root cortex by mycelial growth, followed by oospore formation (Kjøller and Rosendahl, 1998). Upon decay of the root, the oospores are released into the soil where they may remain as an inoculum source for many years (Oloffson, 1984). Neither fungicides, nor resistant or tolerant cultivars are commercially available and this has prompted an interest for biological control of this pathogen. Antagonists, such as the bacteria *Pseudomonas fluorescens* (Bowers and Parke, 1993) and *Burkholderia cepacia* (Heungens and Parke, 2001), have proved effective against *A. euteiches*. However, the only efficient 'control' of the pathogen is to avoid infested fields (Heungens and Parke, 2001). Arbuscular mycorrhizal (AM)-fungi have been shown to positively affect plant health and having antagonistic effects against plant pathogens (Dehne, 1982; Lindermann, 1994; Azcón-Aguilar and Barea, 1996). Other reports, however, show no effect or even an increase in disease severity (St-Arnaud et al., 1995). Pathogen suppression has been ascribed to both physiological changes in mycorrhizal plants and direct interactions between AM-fungi and pathogens. However, the precise understanding of mechanisms is poorly understood (Azcón-Aguilar and Barea, 1996).

In most studies with AM-fungi and *A. euteiches*, biocontrol requires pre-inoculation of mycorrhiza (Rosendahl, 1985; Slezack et al., 1999), although reduction in damage from other pathogens has been reported upon co-inoculation or post-inoculation of

mycorrhiza (Caron et al., 1986). Zoospores added to the epicotyl have constituted the pathogen inoculum in the majority of studies on *A. euteiches*/mycorrhiza interactions. However, it is important to examine the biocontrol efficacy of mycorrhiza upon co-inoculation of oospores, as oospores constitute the natural soil inoculum.

Both indirect and direct methods of quantifying *A. euteiches* are available. Indirect methods such as determination of disease severity index of plants (Parke and Grau, 1992), in terms of, for example, discolouring of the root system, however, might not be directly correlated to *A. euteiches* infection.

Furthermore, disease symptoms may be a delayed response of the proliferation of the pathogen inside the root (Pfender, 1984). A more direct quantification of *A. euteiches* can be obtained by methods based on serology (Slezack et al., 1999; Kraft and Boge, 1994), biochemistry (polyacrylamid gel electrophoresis and densitometry) (Kjøller and Rosendahl, 1997; Bødker et al., 1998), and microscopy after staining with trypan blue (Phillips and Hayman, 1970) or for alkaline phosphatase (Tisserant et al., 1993; Kjøller and Rosendahl, 1998). These direct methods can also be used to quantify AM-fungi. Recently, signature fatty acids have been used to quantify biomass and energy reserves of *A. euteiches* and AM-fungi in roots, using phospholipid fatty acids (PLFAs) and neutral lipid fatty acids (NLFAs), respectively, (Larsen et al., 2000; Larsen and Bødker, 2001). Fatty acid analysis using PLFAs and NLFAs is very laborious, and alternatively whole cell fatty acids (WCFA) analysis representing the sum of all fatty acids present in the microbes can provide a fast and easy way to screen for and quantify *A. euteiches* (Larsen et al., 2000) and AM-fungi (Peng et al., 1993).

The objective of this study was to investigate whether two mycorrhizal species, *Glomus intraradices* and *Glomus claroideum*, have an effect on pea upon co-inoculation of *A. euteiches* oospores. Interactions of these fungi were examined in both root and soil using signature WCFA 16:1ω5c and 14:1ω9.

Materials and methods

Experimental design

The experiment had a randomised three-factorial design with the root pathogen *A. euteiches* as one factor (without and with), mycorrhiza as the second (non-AM, *G. intraradices, G. claroideum*), and harvest time as the third (days 19, 26, and 33). Five replicate pots from each treatment were randomly arranged in temperature-regulated containers providing a constant soil temperature. The pots were rearranged every other day to avoid variations within blocks.

Soil and biological materials

Sandy loam soil from an organic grown field (Snubbekorsgård, Tåstrup, Denmark) was partially sterilised by irradiation (10 kGy, 10 MeV electron beam) and mixed with quartz sand obtaining a ratio of 1 : 3 soil : sand (w/w). Basal nutrients were mixed into the soil in the following amounts (mg kg^{-1} soil): K_2SO_4 (75), $CaCl_2$ (75), $CuSO_4 \times 5H_2O$ (2.1), $ZnSO_4 \times 7H_2O$ (5.4), $MnSO_4 \times H_2O$ (10.5), $CoSO_4 \times 7H_2O$ (0.39), $NaMoO_4 \times 2H_2O$ (0.18), and $MgSO_4 \times H_2O$ (45). The soil : sand mixture had a pH 6.1 and contained 18 mg Olsen P kg^{-1}. Inoculum of the AM-fungi *G. intraradices* Schenck & Smith (BEG 87) and *G. claroideum* Schenck & Smith (BEG 14) were obtained from 3-month-old pot cultures of maize and leek, respectively. The colonised root length in the pot cultures of *G. intraradices* and *G. claroideum* was 88% and 78%, respectively. Crude inoculum of *G. intraradices* and *G. claroideum* containing soil, roots, and spores was homogeneously mixed into the soil : sand mix resulting in a final inoculum concentration of 4% and 6% in the respective mycorrhiza treatments. Oospore-based inoculum of *A. euteiches* Dreschler (ATCC 2016 84), was produced by growing the fungus in oatmeal broth (0.5% oatmeal in demineralised water) at 20 °C in the dark for 8 weeks. Thereafter, the suspension with mycelium and oospores was homogenised for 2 min in a blender and filtered twice through gauze. The suspension was washed with a sterile dilute salt solution (Fuller and Jaworski, 1987) three times by centrifugation at 3000 rpm for 4 min and the oospores were counted in a haemocytometer. Finally, the suspension containing 6.25×10^5 oospores was allowed to dry on 100 g quartz sand, and thereafter mixed homogeneously into the soil : sand mix resulting in a concentration of 390 oospores g^{-1} soil in the *A. euteiches* treatment. A similar amount of quartz sand without oospores was added to the treatments without *A. euteiches*. Seeds of *P. sativum* (cv. Bodil) were surface sterilised in 1.5% NaOCl for 8 min, washed three times in demineralised water, pre-germinated for three days, and sown at a depth of 3 cm with three seeds per 1.25 l pot (12 cm diameter, 14 cm height),

containing 1600 g soil : sand mix, both with and without fungal inoculum. At sowing, 1.6 ml of a dense *Rhizobium leguminosarum* (Risø strain 18a) culture was added to each pea seed. *Rhizobium* was cultured in sterile yeast mannitol broth ($g\,l^{-1}$): $K_2HPO_4 \times 3H_2O$ (0.66), $MgSO_4 \times 7H_2O$ (0.20), NaCl (0.10), D-mannitol (10.0) yeast extract (0.40); and pH was set to 8.0. Pea seedlings were thinned to one per pot after 5 days.

Growth conditions

Plants were maintained in a greenhouse from May to June with no light supplement. Minimum air temperature was 22 °C. The pots were placed in a temperature-regulated container providing a constant soil temperature of 20 °C. Each pot was watered to field capacity at least every second day.

Harvests and analyses

Plants were harvested 19, 26, and 33 days after sowing. At harvest, plants were gently removed from the soil, washed and visually examined for disease severity of the root and epicotyl (discolouration) and shoot (chlorosis and wilting) by scoring percentage area of the respective plant parts with symptoms. The shoot was cut off just above the cotyledons, dried (80 °C for 24 h) and weighed. The root system was weighed and cut into 5 mm pieces, and a subsample of 0.5 g was stained according to the procedure of Kormanick and McGraw (1982), except that acid fuchsin was substituted by a 0.05% solution of trypan blue in lactoglycerol. Total root length, root length with oospores of *A. euteiches* and mycorrhizal structures, as well as percentage root length with these fungal structures were determined by the line-intercept method (Giovannetti and Mosse, 1980). The remaining root system was freeze-dried for 4 days, weighed, and homogenised by vortex-mixing with a steel mill ball in teflon test tubes with liquid nitrogen and kept in the freezer at −80 °C until further processed. Similarly, soil samples without roots were freeze-dried for four days and kept in the freezer.

Whole cell fatty acid extraction was performed according to Sasser (1990) and quantification of the individual fatty acids was done according to Larsen et al. (2001). About 10 mg of the freeze-dried root homogenate or 3 g of freeze-dried soil was used for the extractions. Identification of fatty acid methyl esters was performed using the software package Sherlock Version 3.1 (MIDI Inc.) with the HP Chemstation and a HP5890 CG fitted with a 25 m fused silica capillary column (HP part no.19091B-102) and hydrogen as carrier gas. In roots and soil, WCFA 16:1ω5c was used to estimate mycorrhiza colonisation levels, whereas WCFA 14:1ω9 was used as indicator of *A. euteiches* root infection levels.

Statistics

Analysis of variance, using General Linear Model (GCM), and Least Significant Means analysis were used to analyse data, using SAS 8e (SAS Institute Inc., 1999). All percentage values were arcsine transformed before GLM analysis, and the remaining values were log transformed.

Results

Thirty-three days after sowing, all measured plant parameters, shoot (Figure 1A) and root dry weights (Figure 1B), and root length (Figure 1C) ($P < 0.0001$) were reduced by *A. euteiches* inoculation. At the last harvest, plants inoculated with AM-fungi were less affected by *A. euteiches* inoculation compared to treatments without mycorrhiza inoculation ($P \leq 0.01$). However, shoot dry weight (Figure 1A) and total root length (Figure 1C) of plants with *G. intraradices* were unaffected by *A. euteiches*, in contrast to plants with *G. claroideum* ($P \leq 0.01$). Furthermore, shoot dry weight and total root length of plants with *G. claroideum* were less affected by *A. euteiches* inoculation, than the corresponding non-mycorrhizal plants. Finally, root dry weight of plants with *G. claroideum* and non-AM plants were equally reduced by *A. euteiches* (Figure 1B). In treatments without *A. euteiches*, all measured plant growth parameters were unaffected by mycorrhiza.

Disease severity of shoot and root, caused by *A. euteiches*, was in general lower in mycorrhizal plants than in non-mycorrhizal plants 26 and 33 days after sowing, although the disease severity of the shoot in the third harvest was unaffected by *G. claroideum*. The disease severity of epicotyls was unaffected by mycorrhiza in all harvests (Table 1). In mycorrhizal roots, *A. euteiches* developed a lower percentage of oospores than in the corresponding roots of non-mycorrhizal plants 26 days after sowing ($P = 0.001$) (Figure 2A). At the final harvest, however, percentage oospores was not reduced in mycorrhizal plants. Furthermore,

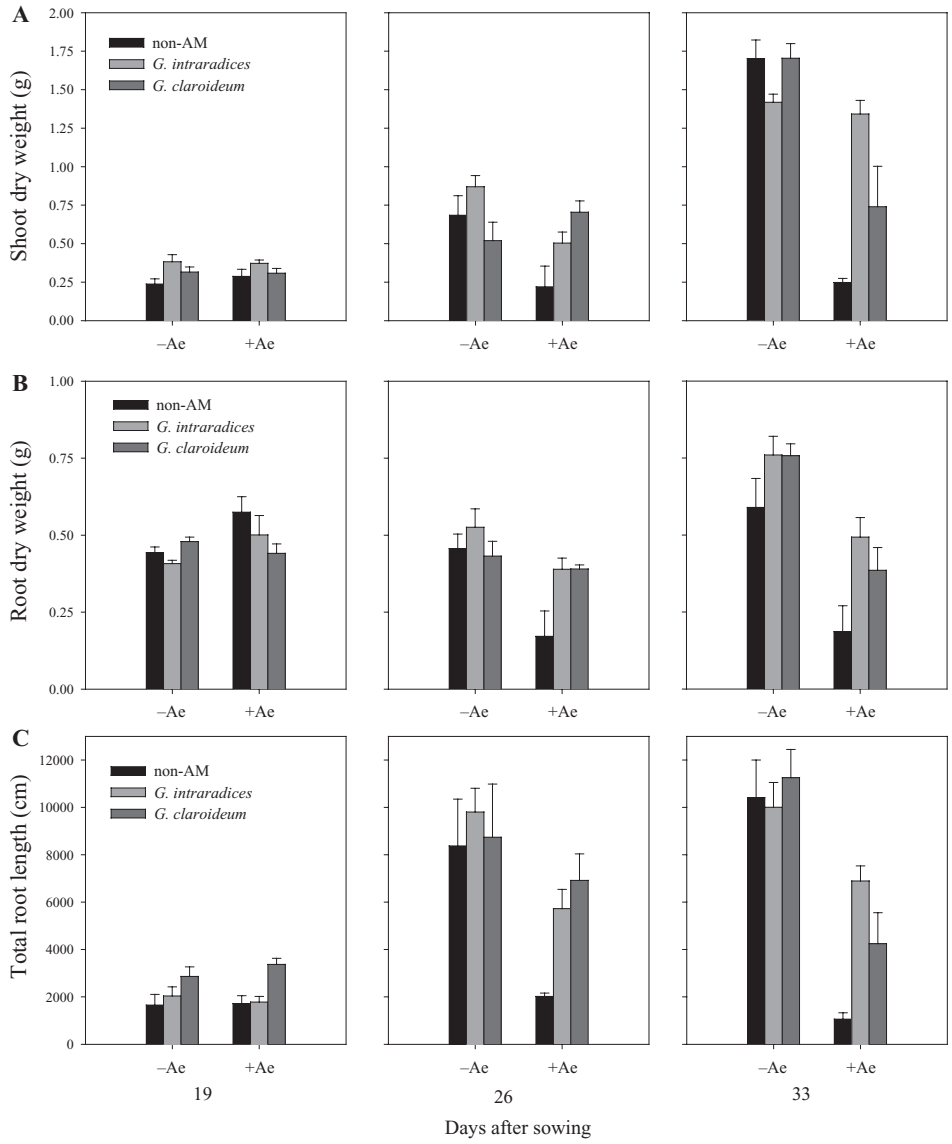

Figure 1. Shoot (A) and root dry weight (B) and total root length (C) of pea inoculated without and with oospores of *A. euteiches* (Ae); without (non-AM) and with the AM-fungi *G. intraradices* and *G. claroideum* 19, 26, and 33 days after sowing and inoculation. Bars indicate standard errors.

the concentration (nmol g^{-1} root dw.) of the *A. euteiches* marker WCFA 14:1ω9 was not reduced in mycorrhizal roots compared to that of non-mycorrhizal roots in any harvests (Figure 2B). No WCFA 14:1ω9 was found in plants without *A. euteiches*.

In the final harvest, 33 days after sowing, root length with oospores was higher in mycorrhizal plants, than in non-mycorrhizal plants ($P = 0.0052$) (Figure 2C). Root length with oospores in non-mycorrhizal plants decreased between the second and third harvest ($P = 0.027$), whereas no difference was observed in mycorrhizal plants between the second and third harvest. Mycorrhizal colonisation was unaffected by *A. euteiches* inoculation (Figure 3A,B). Percent root colonisation of *G. intraradices* was higher than that of *G. claroideum* ($P < 0.0001$), so that 33 days after sowing 78% and 39% root colonisation was found in the respective treatments (Figure 3A).

Table 1. Disease severity index of the epicotyl, root, and shoot of pea inoculated with and without oospores of *Aphanomyces euteiches* (Ae); with and without the arbuscular mycorrhizal (AM) fungi *G. intraradices* (Gi) and *G. claroideum* (Gc) 19, 26, and 33 days after sowing and inoculation

Treatment			Disease severity index		
Harvest (Days)	AM	Ae	Epicotyl (%)	Root (%)	Shoot (%)
19	−	−	0	0	0
	−	+	71.25a	39.50a	0
	Gi	−	0	0	0
	Gi	+	68a	24a	0
	Gc	−	0	0	0
	Gc	+	37.5a	24a	0
26	−	−	0	0	0
	−	+	97.5a	85a	77.5a
	Gi	−	0	0	0
	Gi	+	78a	52b	14b
	Gc	−	0	0	0
	Gc	+	68a	62ab	17b
33	−	−	0	0	0
	−	+	100	99.5a	82.5a
	Gi	−	0	0	0
	Gi	+	100	61b	2.6b
	Gc	−	0	0	0
	Gc	+	100	75.4c	52a
One-way general linear model *p*-values					
19		AM	0.622	0.652	—
26		AM	0.522	0.04	0.001
33		AM	—	<0.0001	0.001

Within a column, numbers from the individual harvests with the same letter are not statistically different (LS-Means analysis).

Similarly, the concentration of the mycorrhiza marker WCFA 16:1ω5c in roots in treatments with both *G. intraradices* and *G. claroideum* was unaffected by *A. euteiches* inoculation and the amount of 16:1ω5c in plants with *G. intraradices* was higher than in plants with *G. claroideum* ($P < 0.0001$) (Figure 3B). Root length with mycorrhiza was in all harvests generally higher in plants with *G. intraradices* than in plants with *G. claroideum* ($P \leq 0.0026$) (Figure 3C).

In soil, 33 days after sowing, the amount of WCFA 16:1ω5c was highest in both of the treatments with *G. intraradices*, and also the *G. claroideum* treatment with *A. euteiches*, than in any other treatment ($P = 0.003$) (Figure 4). These treatments were unaffected by *A. euteiches*. No difference in amount of WCFA 16:1ω5c was found between treatments with *G. claroideum* and non-mycorrhizal controls. Only small amounts of WCFA 14:1ω9 were detected in soil inoculated with *A. eiteiches* (data not shown).

Discussion

To our knowledge, this is the first study to show that co-inoculation of AM-fungi and *A. euteiches* in terms of oospores results in reduced root-rot in pea plants. This is in contrast to other studies on these interactions, which showed that pre-inoculation of mycorrhiza is important to achieve any disease control (Rosendahl et al., 1985; Slezack et al., 2000). Furthermore, this is the first study that compared the influence of two AM-fungi on pea root-rot. Both *G. intraradices* and *G. claroideum* reduced disease incidence of the shoot and root of pea plants. This effect was most pronounced at the final harvest in plants with *G. intraradices*, where the root length and shoots were as large as plants that had not been inoculated with *A. euteiches*.

Disease incidence of the plants in the first two harvests did not differ much, except for non-AM plants with *A. euteiches* being more diseased than the rest in the second harvest. In contrast, greater differences were observed in the final harvest, where focus therefore will remain. Roots inoculated with *A. euteiches* were infected with the same percentage of oospores. However, since *A. euteiches*-inoculated plants with mycorrhiza had fewer above ground disease symptoms, and a much larger plant biomass than the corresponding non-AM plants, this might suggest that tolerance against the pathogen somehow was induced in mycorrhizal plants, as has also been suggested in other studies (Dugassa et al., 1996; Kjøller and Rosendahl, 1996). The underlying mechanisms for this supposed tolerance induction is not clear and needs further examination. However, this adds to the list of possible mechanisms for the control of *Aphanomyces* pea root-rot such as induced resistance (Rosendahl 1985), general physiological changes (Kjøller and Rosendahl, 1996), and competition for nutrients (Larsen and Bødker, 2001). Moreover, increased P nutrition in AM plants does not seem to be involved in suppression of pea root-rot (Bødker et al., 1998). Most likely, the biocontrol activity of AM-fungi against pea root-rot relies on a combination of the various proposed modes of actions.

We cannot completely rule out the possibility that microbes accompanying the mycorrhiza inocula also played a role in the observed biocontrol. However,

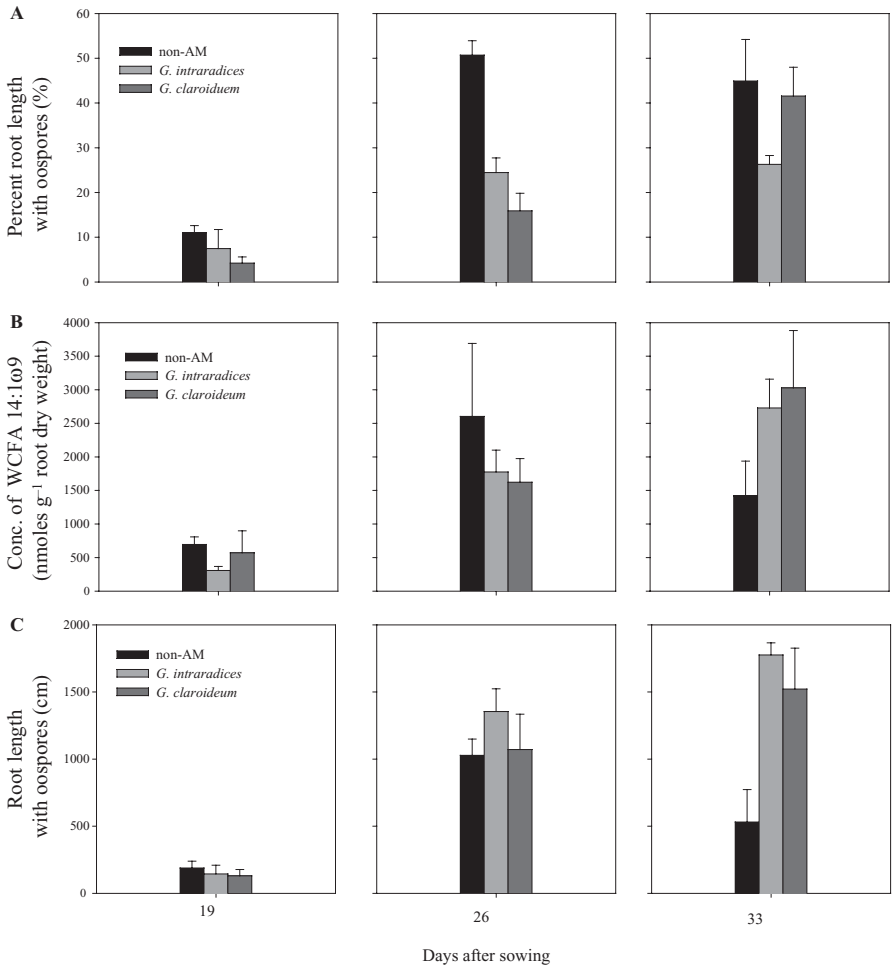

Figure 2. Percentage root length with oospores (A), concentration of WCFA 14:1ω9 (B), and root length with oospores (C) of *A. euteiches*; without (non-AM) and with the AM-fungi *G. intraradices* and *G. claroideum* 19, 26, and 33 days after sowing and inoculation. Bars indicate standard errors.

the microbial communities in the different treatments without *Aphanomyces* did not differ as examined using WCFA profiles (data not presented).

Arbuscular mycorrhizal symbiosis is reported to affect plant water relations by increasing drought resistance and/or tolerance (Augé, 2001). The symptoms of pea root-rot are similar to that of water stressed plants resulting in shoot wilting. Consequently, it could be that some of the mechanisms involved in drought resistance and tolerance are similar to those found in the present experiment where the shoot of plants with *G. intraradices* did not wilt even if the root system almost had the same root-rot development as found in non-mycorrhizal plants. On the other hand, plants with *G. intraradices* had longer roots than that and a greater intra- and extraradical mycorrhiza development than that of plants with *G. claroideum*, which might explain the difference in tolerance induction by these two AM-fungi. The present experiment was conducted under controlled conditions with daily watering providing a sufficient water supply. However, this may not be the case under field conditions where the amount of water may be limited for periods. Consequently, in order to extrapolate the results obtained in the present experiment to field conditions more studies are needed to examine the role of soil water content on the observed tolerance induction. Moreover, more field studies are needed in this area to study disease suppression/tolerance induction by introducing species of AM-fungi selected for this feature.

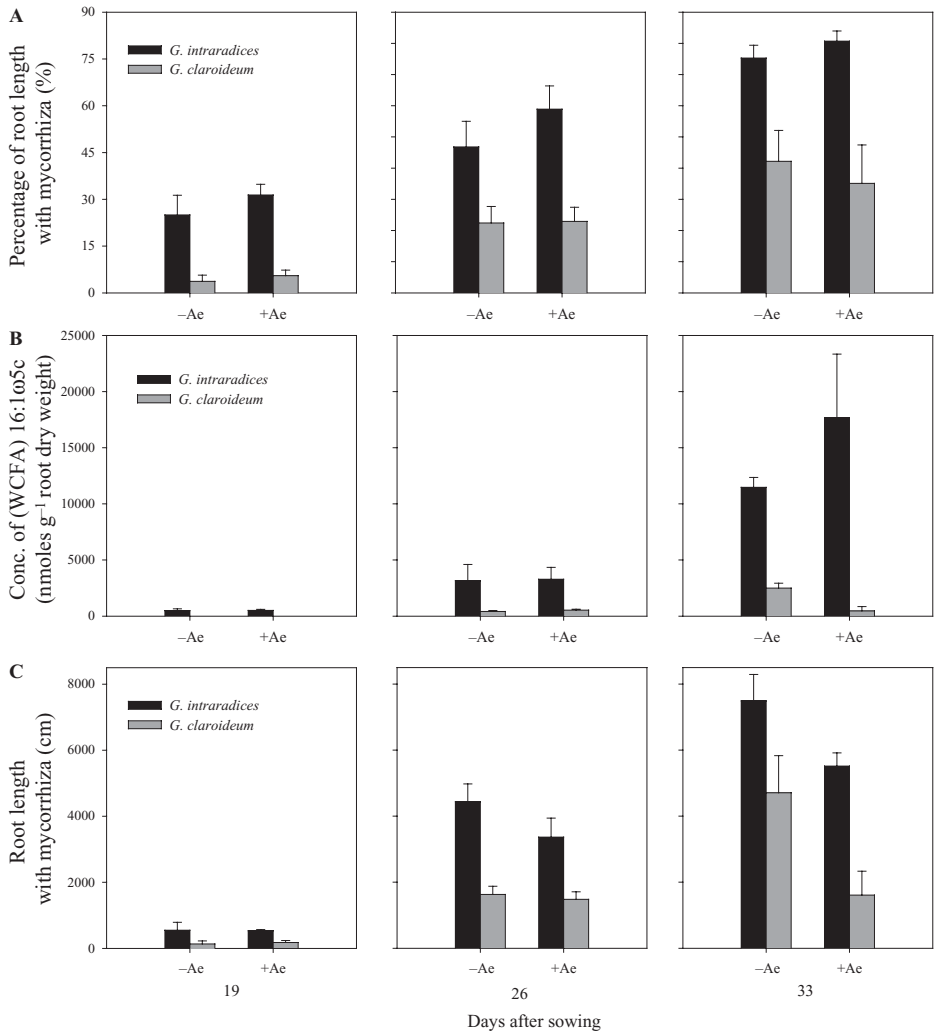

Figure 3. Percentage of root length with mycorrhizal structures (A), concentration of WCFA 16:1ω5c (B) and root length with mycorrhizal structures (C) in pea inoculated with and without oospores of *A. euteiches* (Ae); with the AM-fungi *G. intraradices* and *G. claroideum* 19, 26, and 33 days after sowing and inoculation. Bars indicate standard errors.

Bødker et al. (2002) also used oospores as *A. euteiches* inoculum in a field study to examine the interactions between *A. euteiches* and indigenous populations of AM-fungi reporting a negative correlation between mycorrhiza colonisation and root infection with oospores, but the degree of mycorrhiza colonisation did not correlate with disease severity in terms of root-rot development. In the present study, the soil temperature was kept constant at 20 °C, whereas the soil temperature under field conditions in Denmark is ranging from 5 to 15 °C in the pea growing period from April to July. Hence, in the field situation, oospores of *A. euteiches* and chlamydospores of AM-fungi are interacting at much lower temperatures than used in the present experiment, calling for more controlled experiments examining the influence of soil temperature on the interactions between AM-fungi and *A. euteiches*.

The percentage of mycorrhizal colonisation was measured using the traditional grid-line intersect method (Giovannetti and Mosse, 1980) and similar results were obtained using WCFA 16:1ω5c analysis. Percentage mycorrhizal root colonisation was not reduced by *A. euteiches*. All in all, it seems that in the present study there was no direct interaction between *A. euteiches* and AM-fungi in the form of competition for nutrients or space. This is in contrast to

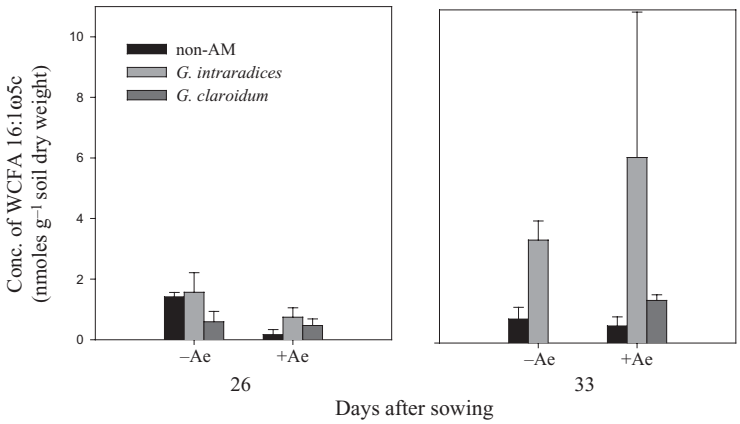

Figure 4. Concentration of WCFA 16:1ω5c in soil inoculated with and without oospores of *A. euteiches* (Ae); without (non-AM) and with the AM-fungi *G. intraradices* and *G. claroideum*, 26 and 33 days after sowing and inoculation. Bars indicate standard errors.

another study on interaction between the AM-fungus *G. mosseae* and *A. euteiches*, where mutual inhibition was observed using signature fatty acids (Larsen and Bødker, 2001). Indeed, the results from the present study show the importance of studying functional compatibility between AM-fungi and host plants, not only in terms of plant nutrients and growth responses, but also in terms in biocontrol activity.

In the final harvest, root length with oospores was higher in treatments with mycorrhiza than without mycorrhiza. However, since root length with oospores in treatments without mycorrhiza decreased between the second and the third harvest, this could suggest that the root had rotted and released oospores to the soil. Nevertheless, we cannot rule out the possibility that mycorrhizal plants host a greater number of oospores per plant due to a larger root system than the non-mycorrhizal plants and thereby increase the soil inoculum potential of the pathogen. These results clearly demonstrate the importance of considering root length measurements when evaluating the biocontrol effect of AM-fungi against root pathogens. Future studies should address this question on the influence of AM-fungi on pathogen inoculum potential.

In conclusion, AM-fungi included in the present study differed in their ability to induce tolerance emphasising the importance of including 'disease control' assessments in future studies on functional compatibility in AM symbioses.

References

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11: 3–42.

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

Bowers JH and Parke JL (1993) Epidemiology of *Pythium* damping-off and *Aphanomyces* root rot of peas after treatment with bacterial agents for biological control. Phytopathology 83: 1466–1473.

Bødker L, Kjøller R, Kristensen K and Rosendahl S (2002) Interactions between indigenous arbuscular mycorrhizal fungi and *Aphanomyces euteiches* in field-grown pea. Mycorrhiza 8: 169–174.

Bødker L, Kjøller R and Rosendahl S (1998) Effect of phosphorus 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.

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

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

Dugassa GD, Alten HV and Shönbeck F (1996) Effects of arbuscular mycorrhiza (AM) on health of *Linum usitatissimum* L. infected by fungal pathogens. Plant and Soil 185: 173–182.

Fuller MS and Jaworski A (1987) *Zoosporic Fungi in Teaching and Research.* Southeastern Publishing Corporation, Athens, GA, USA.

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

Heungens K and Parke JL (2001) Postinfection biological control of oomycete pathogens of pea by *Burkholderia cepacia* AMMDR1. Phytopathology 91: 383–391.

Kjøller R and Rosendahl S (1996) The presence of the arbuscular mycorrhizal fungus *Glomus intraradices* influences enzymatic activities of the root pathogen *Aphanomyces euteiches* in pea roots. Mycorrhiza 6: 487–491.

Kjøller R and Rosendahl S (1997) Polyacrylamide gel electrophoresis (PAGE) and densitometric measurement of enzyme activity of the pea root pathogen *Aphanomyces euteiches* in pea roots. Journal of Phytopathology 145: 253–256.

Kjøller R and Rosendahl S (1998) Enzymatic activity of the mycelium compared with oospore development during infection of pea roots by *Aphanomyces euteiches*. Phytopathology 88: 992–996.

Kormanick PP and McGraw AC (1982) Quantification of vesicular-arbuscular mycorrhiza in plant roots. In: Schenck NC (ed) Methods and Principals of Mycorrhizal Research. American Phytopathological Society, St. Paul, MN, USA, pp. 37–45.

Kraft JM and Boge WL (1994) Development of an antiserum to quantify *Aphanomyces euteiches* in resistant pea lines. Plant Disease 78: 179–183.

Larsen J, Mansfeld-Giese K and Bødker L (2000) Quantification of *Aphanomyces euteiches* in pea roots using specific fatty acids. Mycological Research 104: 858–864.

Larsen J and Bødker L (2001) Interactions between pea root-inhabiting fungi examined using signature fatty acids. New Phytologist 149: 858–864.

Linderman RG (1994) Role of VAM fungi in biocontrol. In: Pfleger FL and Linderman RG (eds) Mycorrhizae and Plant Health. American Phytopathological Society, St. Paul, MN, USA, pp. 1–25.

Oloffson J (1984) Sjukdomar och skadedjur på ärter. *Nordisk Jordbrugsforskeres Forening.* Rapport Nr. 15, Ärtodling 26: 1–8.

Papavizas C and Ayers WA (1974) *Aphanomyces* species and their root rot diseases in pea and sugarbeet. US Department of Agricultural Technical Bulletin 1485: 1–158.

Parke JL and Grau CR (1992) *Aphanomyces*. In: Singleton LL, Mihail JD and Rush CM (eds) Methods for Research on Soilborne Phytopathogenic Fungi. The Phytopathological Society, St. Paul, MN, USA, pp. 27–30.

Peng S, Eissenstat D, Graham JH, Williams K and Hodge NC (1993) Growth depression in mycorrhizal citrus at high-phosphorus supply. Analysis of carbon cost. Plant Physiology 101: 1063–1071.

Pfender WF (1984) Aphanomyces root rot. In: Hagedorn DJ (ed) Compendium of Pea Diseases. The American Phytopathological Society, St. Paul, USA, pp. 25–28.

Phillips JM and Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi and for rapid assessment of infection. Transactions of the British Mycological Society 55: 158–160.

Rosendahl S (1985) Interactions between the vesicular-arbuscular mycorrhizal fungus *Glomus fasciculatum* and *Aphanomyces euteiches* root rot of peas. Phytopathologishe Zeitschrift 114: 31–40.

Sasser M (1990) Identification of bacteria through fatty acid analysis. In: Clement Z, Rudolph K and Sands DC (eds) Methods in Phytobacteriology. Akademiai Kiado, Budapest, pp. 199–203.

Slezack S, Dumas-Gaudot E, Rosendahl S, Kjøller R, Paynot M, Negrel J and Gianinazzi S (1999) Endoproteolytic activities in pea roots inoculated with the arbuscular mycorrhizal fungus *Glomus mosseae* and or *Aphanomyces euteiches* in relation to bioprotection. New Phytologist 142: 517–529.

Slezack S, Dumas-Gaudot E, Paynot M and Gianinazzi S (2000) Is a fully established arbuscular mycorrhizal symbiosis required for bioprotection of *Pisum sativum* roots against *Aphanomyces euteiches*? Molecular Plant–Microbe Interactions 13: 238–241.

St-Arnaud M, Hamel C, Caron M and Fortin JA (1995) Endomychorrhizes VA et sensibilité des plantes aux maladies: Synthèse de la littérature et des mécanismes d'interaction potentiels. In: Fortin JA, Charest C and Piché Y (eds) La symbiose mycorrhizienne: état des connaissances. ORTIS, Frelsghburg, Québec, Canada, pp. 51–87.

Tisserant B, Gianinazzi-Pearson V, Gianinazzi S and Gollote A, (1993) *In planta* histochemical staining of fungal alkaline phosphatase activity for analysis of efficient arbuscular mycorrhizal infections. Mycological Research 97: 245–250.