Journal of Ecology
British Ecological Society

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Journal:	*Journal of Ecology*
Manuscript ID:	JEcol-2009-0266.R2
Manuscript Type:	Standard Paper
Date Submitted by the Author:	
Complete List of Authors:	Sikes, Benjamin; University of Guelph, Integrative Biology Cottenie, Karl; University of Guelph, Integrative Biology Klironomos, John; University of Guelph, Integrative Biology
Key-words:	arbuscular mycorrhizal fungi, Fusarium oxysporum, mycorrhizal function, mycorrhizal identity, pathogen protection, plant-soil interactions, root architecture

Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas

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Summary

1. A major benefit of the mycorrhizal symbiosis is that it can protect plants from below-ground enemies, such as pathogens. Previous studies have indicated that plant identity (particularly plants that differ in root system architecture) or fungal identity (fungi from different families within the Glomeromycota) can determine the degree of protection from infection by pathogens. Here we test the combined effects of plant and fungal identity to assess if there is a strong interaction between these two factors.
2. We paired one of two plants *(Setaria glauca*, a plant with a finely branched root system and *Allium cepa*, which has a simple root system) with one of six different fungal species from two families within the Glomeromycota. We assessed the degree to which plant identity, fungal identity, and their interaction determined infection by *Fusarium oxysporum*, a common plant pathogen.
3. Our results show that the interaction between plant and fungal identity can be an important determinant of root infection by the pathogen. Infection by *Fusarium* was less severe in *Allium* (simple root system) or when *Setaria*

(complex root system) was associated with a fungus from the family Glomeraceae. We also detected significant plant growth responses to the treatments; the fine-rooted *Setaria* benefited more from associating with a member of the family Glomeraceae, while *Allium* benefited more from associating with a member of the family Gigasporaceae.

4. *Synthesis*. This study supports previous claims that plants with complex root systems are more susceptible to infection by pathogens, and that the arbuscular mycorrhizal symbiosis can reduce infection in such plants – provided that the plant is colonized by a mycorrhizal fungus that can offer protection, such as the isolates of *Glomus* used here.

Key-words: arbuscular mycorrhizal fungi, *Fusarium oxysporum*, mycorrhizal function, mycorrhizal identity, pathogen protection, plant-soil interactions, root architecture

Introduction

The arbuscular mycorrhizal (AM) symbiosis is widespread among vascular plants; its benefit to plants, however, can vary widely. Factorial combinations of different plants and fungi have experimentally verified that a ‘continuum of benefit’ occurs from parasitism to mutualism (Johnson *et al.* 1997; Klironomos 2003), where benefit is typically quantified by determining the difference in growth between plants colonized with a particular fungus compared to those without the fungus. Where on this continuum a specific mycorrhizal association falls is based on 1) the needs of the plant and 2) the ability of the fungus to perform a needed function. Placing a specific AM fungus on this continuum may be more complicated than originally anticipated. Evidence is mounting that AM fungi are multifunctional, yet we know little about the determinants of these different functions (Newsham *et al.* 1995b).

While the main role of AM fungi in facilitating phosphorus uptake has been supported in both field and greenhouse experiments (Bolan 1991; Smith & Read 1997), plants with AM fungi can also show improved water relations, reduced uptake of heavy metals and increased protection from pathogens (summarized in Newsham *et al.* 1995b). In some cases these ‘alternate’ functions appear to be the primary benefit a plant receives from the symbiosis (Borowicz 2001; Newsham *et al.*; 1995a; Singh *et al.* 2000; Herre *et al.* 2007; Fitter, 1985). Which particular mycorrrhizal function is more important may be driven by environmental factors pressuring the plant. For example, when the plant host is faced with many root pathogens but nutrients are relatively abundant, plants may benefit more from pathogen protection. When pathogen loads are low and P is limiting (as in many greenhouse experiments) the primary benefit of the AM association to the plant may be acquisition of P. Under these two scenarios the same fungus would have very different functions, however, net benefit for the plant (increased biomass or fitness) could be similar. Recent evidence indicates that these two particular functions differ among AM fungi and correlate with their broader phylogeny (Maherali & Klironomos 2007). This result indicates that AM fungi that are best able to protect plants from pathogens would be more beneficial under conditions of high pathogen abundance. In the absence of pathogens, these AM fungi may have a negative effect on plant growth (parasitism) due to their demand for plant photosynthate. Likewise, AM fungi that are best equipped for P acquisition may be poor partners when P concentrations are not limiting (Johnson 1993). As a result, while two different fungi could perform the same function, one fungus is more beneficial under certain conditions. In determining a particular mycorrhizal function, both plant need and fungal ability are not mutually exclusive and are likely acting simultaneously. Thus, our goal is to test if both factors interact to determine a specific mycorrhizal function and if so, to what degree each determines plant benefit.

Evidence for plant-based determinants of mycorrhizal functioning is shown in the research of Newsham *et al*. (1995; 1995b) who illustrated that a plant with a highly branched, fine root system was less dependent on mycorrhizas for nutrient acquisition. Highly branched roots should be more susceptible to infection by soil pathogens because of increased numbers of meristems and lateral roots where pathogenic fungi can invade; therefore, these plants should benefit more from mycorrhiza-mediated pathogen protection. *Vulpia ciliata* ssp. *ambigua*, a plant with highly branched roots, showed reduced negative effects from both *Fusarium oxysporum* and *Embellisia chlamydospora* when inoculated with a single *Glomus* species (Newsham *et al.* 1995a). Earlier research by the same group showed that *Hyacinthoides non-scripta* is obligately dependent on AM fungi for its P uptake, likely due to its poorly branched root system (Merryweather and Fitter 1995). Newsham *et al.* (1995b) hypothesize that this poor branching would also make this species less vulnerable to infection by soil pathogens. While susceptibility to pathogens may vary among plants, their roots may be colonized by mycorrhizal fungal partners that differ in their ability to protect the plants.

While many studies have now reported that plant growth benefit depends partly on the identity of AM fungal symbionts (Klironomos 2003; Sanders and Fitter 1992; van der Heijden *et al*. 1998), recent evidence indicates that even the main function of the association may differ depending on the fungi involved. Both pathogen protection and P uptake can vary widely depending on the AM fungal symbiont (Garmendia *et al*. 2004; Vogelsang *et al*. 2006). Maherali & Klironomos (2007) showed evidence that this variation in mycorrhizal function is related to the broader phylogeny of the phylum Glomeromycota. In their research, AM fungi from the Family Glomeraceae were more effective than AM fungi from the Family Gigasporaceae at reducing infection by either *F. oxysporum* or a *Pythium* sp. in *Plantago lanceolata*. In contrast, members of the Gigasporaceae were more effective than those of the Glomeraceae at enhancing P uptake by plants. These functional differences may be a result of the distinct life-history strategies found in these two AM fungal families. The family Gigasporaceae is typified by slow-colonizing species with hyphae concentrated outside the plant root, while members of the

Glomeraceae colonize rapidly and usually have hyphae concentrated within the root (Hart & Reader 2002; Maherali & Klironomos 2007). While identity of AM fungi could be a determinant of mycorrhizal functioning, whether that association is beneficial (and possibly sustained) depends on whether the plant host needs that given function.

In this study we test the hypotheses proposed by Newsham *et al.* (1995a) and Maherali & Klironomos (2007) – whether a single mycorrhizal function, pathogen protection, is determined by a) the identity of plants with contrasting root architectures b) the identity of the family of AM fungi with which they are associated, and c) their interaction. We then examine how plant benefit differs depending on these interactions. Finally, we test one potential mechanism of pathogen protection by AM fungi.

If the plant drives the function, then we predict that the coarse-rooted plant will be protected more from our pathogen than the fine-rooted plant, regardless of the identity of their mycorrhizal partners. Alternatively, if the fungus drives the function, then we predict that plants partnered with fungal species from the Glomeraceae will have lower pathogen levels than plants associated with species from the Gigasporaceae, regardless of plant host identity. Finally, it is also likely that pathogen protection is driven by the interaction between plant and fungal identity. In such a scenario, we predict that pathogen infection is reduced by a member of the Glomeraceae, but only in highly susceptible plants.

For the plant growth benefit, we predict that 1) the plant with more complex root architecture will benefit most from AM fungi in the Glomeraceae, because the plant has a root structure susceptible to pathogens and species from the Glomeraceae are better at pathogen protection and 2) a plant with a simple root architecture will not benefit much from pathogen-protecting species (Glomeraceae) because of its low susceptibility to *F. oxysporum*, but will benefit most from members of the Gigasporaceae because of their greater potential to aid with nutrient uptake.

Using our data we were also able to test one of the proposed mechanisms for pathogen protection by AM fungi (Azcon-Aguilar & Barea 1996). Colonization by AM fungi may compete with soil pathogenic fungi for infection sites, thus affording the plant protection (Azcon-Aguilar & Barea 1996; Dehne 1982). Increased levels of root colonization by members of the Glomeraceae could more effectively reduce pathogen infection sites (Hart & Reader 2002a; Maherali & Klironomos 2007). Therefore, we predict that 1) Glomeraceae species should have greater internal root colonization than Gigasporaceae species and 2) after accounting for differences between plants and AM fungal families (the original treatment), the severity of *F. oxysporum* infection should be negatively correlated to the degree of AM fungal colonization.

Materials and Methods

Mycorrhizal fungal inoculum

Mycorrhizal spores were isolated from soils collected at the Long-Term Mycorrhizal Research Site (LTMRS) at the University of Guelph, Guelph, Ontario, Canada (43°32’30N”,80°13’00”W). This site is an old-field meadow, dominated by forbs and grasses, that has been left undisturbed for more than 40 years. All six fungal isolates used in this experiment collected from the LTMRS and maintained in greenhouse pot cultures using *Allium porrum* (leek) as a host. We used the following AM fungal isolates in this experiment: *Glomus intraradices* Schenk & Smith, *Glomus etunicatum* Becker and Gerdemann, *Glomus clarum*, *Gigaspora margarita*, *Gigaspora gigantea*,and *Scutellospora pellucida* (Klironomos *et al.* 2000).

Fusarium inoculum

Fusarium oxysporum was also isolated from LTMRS soil. Soil suspension was added to Malt Extract Agar (MEA), and a variety of fungal colonies grew as a result. Several colonies of *F. oxysporum* were identified and re-cultured on MEA. Three colonies were pooled and used in the experiment. Prior to adding *F. oxysporum* to the experimental units, fungal material (hyphae and spores) was inoculated onto malt extract agar in a one-litre bottle. The fungi were left to grow for up to six weeks, until the colonies were covered with spores. Spores were then washed from the bottle and spore concentrations were determined using a haemocytometer.

<u>Soil Pre-treatments</u>

Soils consisted of 70% sand and 30% LTMRS field soil, both sterilized by autoclaving. The resulting soil mixture contained the following, NH_4= 3.8 mg kg^{-1}; NO_3 =2.7 mg kg^{-1}; P=2.1 mg kg^{-1}; K=31mg kg^{-1}; pH= 7.6. Soils were thoroughly homogenized and used to fill 1.5-L pots. To each pot we added approximately one gram of root inoculum (chopped roots) from pot culture plants either infected with a specific AM fungal isolate or not infected with AM fungi as a control. Root inoculum was buried approximately 1 cm below the soil surface. Each pot also received a microbial wash derived from all the pot culture soils to control for any background contaminants that are introduced with pot culture material. The microbial wash was the filtrate of pot culture soils suspended in de-ionized water and passed through a 20µm sieve. Approximately 50 mL of filtrate was added to each pot.

Experimental Design

Pots were arranged in a complete randomized design on a greenhouse bench. There were 8 *F. oxysporum*–AM fungal treatment combinations (no fungal additions (control), *F. oxysporum* only (F only), *F. oxysporum* + one of the six AM fungal species (e.g. F+Gl.intr)) for each plant species (16 in total) and 10 pots per treatment combination for a total of 160 replicates.

Plants and Treatment timing

Allium cepa (Liliaceae) and *Setaria glauca* (Poaceae) were used as plant hosts because they occur locally, form arbuscular mycorrhizas and have contrasting root architectures.   Seeds of *A. cepa* were collected from plants that were introduced to a recently disturbed meadow adjacent to the LTMRS. Seeds of *S. glauca* were collected from a weedy roadside community next to the LTMRS. All plant seed was moistened with sterile distilled water and placed at 4 °C for 2 months prior to being introduced to the greenhouse pots. Three seeds of either *A. cepa* or *S. glauca* were germinated in each pot and then seedlings were thinned to a single individual per pot. Plants were watered daily for the first two weeks and subsequently watered every two days. After the first four weeks, plants were fertilized weekly with 20 mL half-strength Hoagland's solution (the full-strength solution contained (mol m^{-3}): $MgSO_4$, 2.0; $Ca(NO_3)_2$, 5.0; KNO_3, 5.0; $NH_4H_2PO_4$, 1.0, together with micronutrients and iron-EDTA) because they showed signs of nutrient deficiency in their leaves. They were grown for five months to give AM fungi maximum time to establish and then inoculated with either a water control or approximately 1,000,000 spores of *Fusarium oxysporum* in a water suspension applied directly to plant roots using a syringe (we commonly retrieve such spore concentrations in the rhizosphere of field plants from anamorphic ascomycete fungi, *Fusarium* spp. included). Plants were then grown for another month and harvested. After we determined wet weight, a root sample was taken for staining of fungal structures. Plants were then oven-dried at 60 °C for two days and weighed again to determine total plant dry weight. Dry weights were adjusted for the roots that were removed for staining.

<u>Percentage colonization</u>

Roots were stained with Chlorazol Black E (Brundrett et al., 1984), and percentage colonization by *F. oxysporum*, or AM fungi, was determined using the magnified intersect method (McGonigle *et al.* 1990). We randomly selected eighteen (2-cm long) root fragments from each pot and mounted them onto two glass slides. For each experimental unit we assessed the presence of *F.oxysporum* and AM fungal structures at 150 intersections. *F. oxysporum* was distinguished from AM fungi by the presence of linear, septate hyphae in the former compared to non-septate (or irregularly septate), knobby hyphae in the latter.

Statistical Analysis

To test for main effects of plant and fungal identity (and their interaction) on pathogen protection, we used Analysis of Variance (ANOVA) where the percentage of root length infected by *F. oxysporum* was the dependent variable and plant species and AM fungal species nested within AM fungal family (Glomeraceae and Gigasporaceae) were independent factors. Because AM fungal species was not a statistically significant factor, we removed it from the model and re-analyzed the data. We used Tukey *post hoc* tests to compare *F. oxysporum* infection between individual plant and AM fungal family combinations. Within each plant species we used ANOVA and Tukey *post hoc* tests to compare differences in *F. oxysporum* infection between *F. oxysporum* only treatments and each *F. oxysporum*–AM fungal treatment.

For plant biomass, we first wanted to determine if infection by *F. oxysporum* affected plant growth. We used regression analysis to test whether *F. oxysporum* infection was correlated with plant biomass overall and for each plant separately (for all fungal addition treatments). For plant biomass by treatments, we used a similar ANOVA approach as for the pathogen infection analyses to test for differences between plants partnered with different AM fungal families. Within each plant species, we also tested for differences in biomass between each fungal treatment (plants without infection, infected with *F. oxysporum* only and each AM fungal treatment) and used Tukey tests to compare individual treatments.

For differences in AM fungal colonization, we used ANOVA to test if the percentage root length of AM fungal colonization differed between plants and AM fungal families and subsequently among *F. oxysporum*–AM fungal treatments with ANOVA and Tukey *post hoc* tests as above. We then used regression analysis to determine if AM fungal colonization was significantly correlated with the residual variation in *F. oxysporum* infection from our original plant and fungal identity model. We used Bonferroni corrections to account for multiple tests. Percentage colonization data for both *F. oxysporum* and mycorrhizal species were arcsine, square root-transformed to increase their conformance to normality. Data were analysed using the R program (http://www.cran.r-project.org/). Graphical representations were constructed in R using the lattice plotting package (Sarkar 2008). For figures, percentage colonization data was not transformed.

Results

Overall, we found significant effects of both plant identity ($p<0.0001$, $F_{1,116}=71.82$) and fungal family identity ($p<0.0001$, $F_{1,116}=65.63$) on pathogen protection measured as infection by *F. oxysporum*, as well as a significant interaction between these factors ($p<0.0001$, $F_{1,116}= 80.16$; Figure 1). For *A. cepa*, the percentage of root length infected by *F. oxysporum* hyphae was small when inoculated with *F. oxysporum* alone ($\bar{x}$ = 15.2%). We detected no difference in the percentage of *F. oxysporum* infection between *A. cepa* roots inoculated with either AM fungal family ($p>0.5$). In addition, there were no significant differences in percentage *F. oxysporum* infection levels between *A. cepa* roots inoculated with *F. oxysporum* only and those inoculated with both *F. oxysporum* and any of the AM fungi ($p>0.5$ for all pairwise comparisons; Fig. 1). In contrast to *A. cepa*, percentage root infection by *F. oxysporum* was high in roots of *S. glauca* inoculated only with *F. oxysporum* ($\bar{x}$ =48.7%). Percentage root infection by *F. oxysporum* was equally severe in *S. glauca* plants inoculated with *F. oxysporum* and members of the Gigasporaceae ($\bar{x}$ = 49.3%), but was significantly less when inoculated with members of the Glomeraceae ($\bar{x}$ = 15.5%; $p<0.0001$; Fig. 1). Within *S. glauca*, plants inoculated only with *F. oxysporum* had similar infection levels to those inoculated with *F. oxysporum* and any member of the Gigasporaceae ($p>0.5$ for all pairwise comparisons), but infection in these treatments was significantly greater than in plants inoculated with any member of the Glomeraceae ($p<0.0001$ for all pairwise comparisons; Fig. 1).

Overall, we found a significant negative correlation between *F. oxysporum* infection and total plant biomass ($p<0.0001$, $R^2=0.299$), but this relationship was strong in *S. glauca* ($p<0.0001$, $R^2=0.570$) and did not hold for *A. cepa* ($p>0.5$, $R^2<0.001$).

Plant biomass was strongly influenced by the fungal treatments. Although we did not detect significant differences based on plant identity ($p=0.317$, $F_{1,116}=1.095$), we did find a significant effect of fungal family ($p<0.0001$, $F_{1,116}=37.31$) as well as a significant interaction between these factors ($p<0.0001$, $F_{1,116} = 187.69$) on total plant biomass (Fig. 2). Overall, the biomass of *A. cepa* was significantly greater when inoculated with *F. oxysporum* and members of the Gigasporaceae than with members of the Glomeraceae ($p<0.0001$), but with some variation within fungal families. For *A. cepa* plants, there was no significant difference in plant biomass among those individuals that were not inoculated with any fungi (x̄ = 1.81g), those inoculated only with *F. oxysporum* (x̄ = 1.92g), and those inoculated with both *F. oxysporum* and either *Glomus intraradices* (x̄ = 2.25g) or *Glomus clarum* (x̄ = 2.17) ($p>0.05$ for all pairwise test comparisons). Plants inoculated with both *F. oxysporum* and *Glomus etunicatum* (x̄ = 2.64) had significantly more biomass than un-inoculated plants ($p<0.05$), but had similar biomass to *F. oxysporum*-only plants, plants partnered with other members of the Glomeraceae and those partnered with members of the Gigasporaceae ($p>0.05$ for all, Fig. 2). *Setaria glauca* plant response was reversed, having significantly greater biomass when inoculated with *F. oxysporum* and members of the Glomeraceae than plants inoculated with *F. oxysporum* and members of the Gigasporaceae ($p<0.0001$). There was no significant variation within fungal families. Biomass of *S. glauca* plants inoculated with *F. oxysporum* alone (x̄ =1.46g) was not significantly different from plants inoculated with both *F. oxysporum* and any member of the Gigasporaceae (x̄ = 1.61g) ($p>0.5$ for all pairwise comparisons), whereas un-inoculated plants (x̄ = 3.65) and those inoculated with both *F. oxysporum* and members of the Glomeraceae (x̄ =3.68g) were significantly higher ($p<0.0001$ for all pairwise comparisons; Fig. 2).

There was a strong interaction between plant identity and AM fungal family on the extent of AM fungal colonization ($p<0.0001$, $F_{1,116}= 31.39$) as well. Although both plants had significantly higher AM fungal colonization by members of the Glomeraceae ($p<0.0001$, $F_{1,116}= 213.41$), in *A cepa* the difference between fungal families was much greater than in *S. glauca* (Fig. 3). *Allium cepa* plants inoculated with species from the Glomeraceae ($\bar{x}$ = 54.7%) were significantly more colonized than those inoculated with members of the Gigasporaceae ($\bar{x}$ = 16.7%)($p<0.0001$). For *S. glauca* plants, although the extent of colonization varied more by particular mycorrhizal species (Fig. 3), overall the two fungal families were still significantly different ($p<0.0001$, Glomeraceae $\bar{x}$ = 24.6%; Gigasporaceae $\bar{x}$ = 10.1%; Fig. 3). Pairwise comparisons between individual fungal species are shown in Fig. 3.

We did not find a significant correlation between the severity of *F. oxysporum* infection and the degree of AM fungal colonization after accounting for variation due to plant and fungal family identity ($p=0.454$, $R^2=0.004$)

Discussion

Our data supports the Newsham *et al.* (1995b) hypothesis that plant identity can determine the degree to which AM fungi can protect plant roots from pathogens. The two tested plants strongly differ in their root architecture, similar to those compared in Newsham *et al.* (1995b). The AM fungal partner played a larger role in protecting the root from a pathogen in the fine-rooted plant compared to the coarse-rooted plant. However, in addition our data also support the hypothesis that the identity of the AM fungi influences the ability of the mycorrhiza to reduce pathogen infection as previously demonstrated by Maherali & Klironomos (2007). More importantly, we found that the interaction of these two factors was a major determinant of how successful a common pathogen was at infecting a plant's root system.

While our data did not explicitly address the mechanism of pathogen protection by AM fungi, we were able to test if higher levels of AM fungal colonization decreased infection by our pathogen possibly by limiting infection sites (Azcon-Aguilar & Barea 1996; Dehne 1982; Maherali & Klironomos 2007). Members of the Glomeraceae had higher percentage colonization and resulted in lower pathogen infection in our susceptible plant. However, the severity of pathogen infection in our study was better explained by the interaction of plant and fungal family identity than the degree of AM fungal colonization.

In this study we focused on a specific mycorrhizal function (pathogen protection). However, our data indicate that a trade-off may exist in AM fungi among their different functions. While AM fungal-mediated pathogen protection is typically viewed as an auxiliary function, our study and others indicate that it can have strong repercussions for plant performance (Newsham *et al.* 1995b; Klironomos 2002; Mitchell & Power 2003). Studies suggest that negative interactions between plants and their pathogens may be a determinant of plant community structure (Klironomos 2002; Mitchell & Power 2003), however, more research is needed in this area. Thus, the ability of AM fungi to protect against such negative interactions may be equally important for plant communities. However, little is known about what edaphic factors influence AM-mediated pathogen protection or the relative contribution of different AM functions to plant communities. Under field conditions, plants are typically colonized by multiple AM fungi at once (Daft 1983; Merryweather & Fitter 1998), but we know little about how functional complementarity of AM fungi differs between these communities (Jansa *et al.* 2008; Maherali & Klironomos 2007; Lekberg *et al.* 2007). Our data indicate that the ability to protect plants from pathogens differs at the family level; therefore colonization by multiple species in the same family may be redundant. However, we tested only a single pathogen and a few AM fungi, so functional variation between species (within a family) may occur for other pathogens or using more mycorrhizal species (although a larger group of AM fungi and two pathogens were tested in Maherali & Klironomos (2007) with consistent family-level divergence in pathogen protection by AM fungi). Alternatively, colonization by multiple fungal species within the same family may represent differences in colonization timing rather than functional niche complementarity.

We recognize that a plant's root architecture and its partnerships with mycorrhizas are not independent factors in nature. Indeed, nutrient limitation can induce changes in plant root morphology like increasing fine root hairs, but association with AM fungi can be an alternate solution (Hetrick 1991). There is evidence that colonization by AM fungi can either stimulate or inhibit root branching (Hetrick *et al.* 1988; Hetrick *et al.* 1991; Price *et al.* 1989; Olah *et al.* 2005). Reduced branching is typically attributed to a decreased ability for plants to directly take up nutrients. However, it could also be a change in root morphology that is triggered by AM fungal colonization resulting in a decrease in potential infection sites for pathogens. Mycorrhizal-mediated changes in plant root morphology for plants may be similarly based on both the degree of root plasticity for a given plant and the identity of its fungal partner. Exploring how changes in plant root architecture due to fungal colonization affect multiple AM functions may modify our understanding of below-ground feedbacks in this symbiosis.

The current study was conducted using two plant species with distinct root system architecture (highly-branched versus simple roots). An obvious follow-up question is whether other plant species with a wide range of root system architectures show similar responses to mycorrhizal colonization. In future studies, measures of multiple functions at the same time (e.g. pathogen protection and P uptake) could provide insight on trade-offs among different fungi. In addition, while we used only a single pathogen, multiple pathogens could be used to determine how broadly protection occurs and to better mimic a plant's normal soil environment. Timing of inoculations may be a key determinant of AM fungal-mediated pathogen protection particularly if priority effects determine the outcome of the interaction (Kennedy and Bruns 2005). In our study, plants were inoculated with AM fungi for five months prior to any pathogen addition, which ensured the AM fungi had colonized but also likely gave them an advantage. A main reason for this timing discrepancy is that we exposed the plants to AM fungi in the form of chopped mycorrhizal roots (a highly disturbed fungal mycelium), which is very different from the more intact mycelial network that plants would be exposed to in the field. It is likely that plants are connected to an extensive and functional network very quickly in the field, even with slow-growing fungi from the Gigasporaceae (Hart & Reader 2002). Nonetheless, differing the timing of AM fungi and pathogen infection may provide further insight on the mechanisms of the observed interactions.

Along with a few additional taxa, Maherali & Klironomos (2007) used the same AM fungal isolates as we did in the present study. It is interesting to note that in both studies similar responses in pathogen protection were observed, despite using different plant species (*Plantago lanceolata* was used in the former). However, plant biomass responses to the AM fungi were very different between the studies. This is not surprising considering the strong plant x fungal genotype interaction in plant growth response that has been observed in other studies (e.g. Klironomos 2003; van der Heijden *et al.* 1998).

In conclusion, it is becoming increasingly clear that AM associations are multifunctional, as proposed by Newsham *et al.* (1995). In this study we show that for one function (pathogen protection), both plant identity and fungal identity can determine the outcome of the association, and that these two factors interact. Further work should focus on assessing the relative importance of different mycorrhizal functions in natural systems and the specific plant and fungal traits involved.

Acknowledgements

We would like to thank A. Stachowicz for technical assistance and the Natural Sciences and Engineering Research Council of Canada for financial assistance. We also thank J. Powell and two anonymous reviewers for helpful comments on a previous version of the paper.

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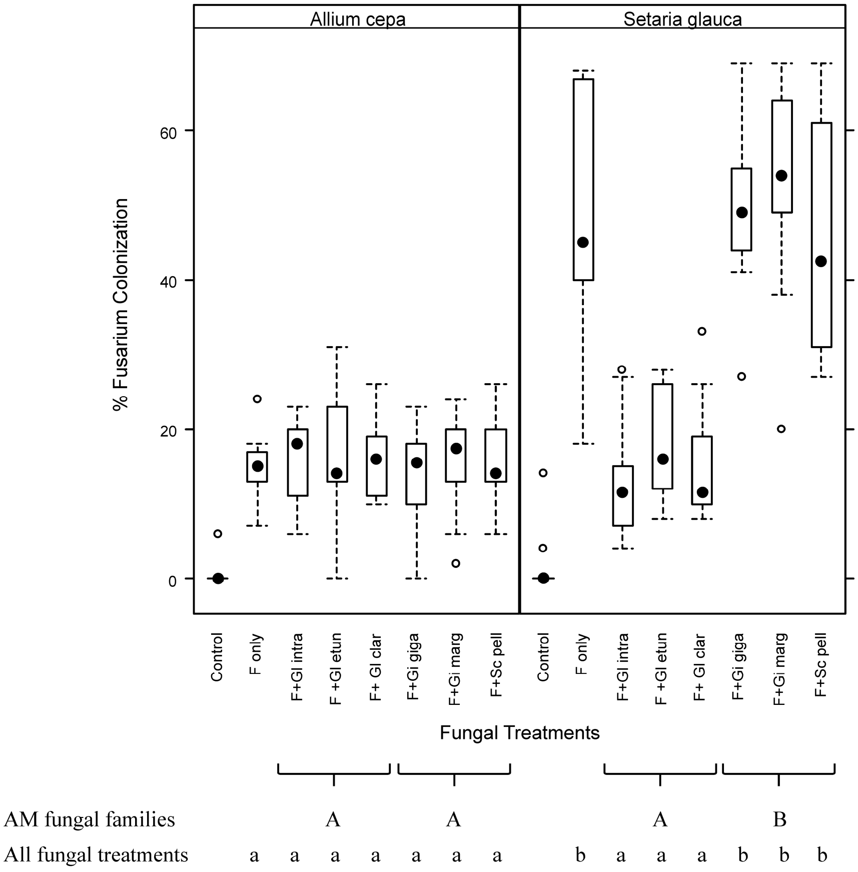
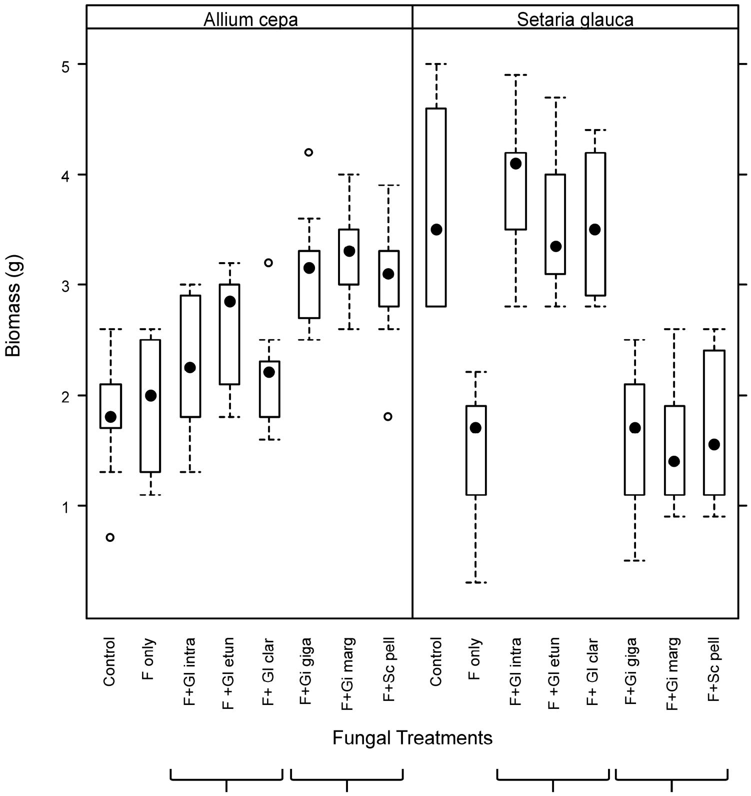


Figure 1: The effect of different fungal additions on *Fusarium oxysporum* infection in *Allium cepa* (coarse, simple roots) or *Setaria glauca* (fine, branched roots). Fungal treatments are as follows: Control - no fungi added, F only - F. oxsporum only added, F+sp - effect of addition of *F. oxysporum* and the species indicated (Gl. intra.= *Glomus intraradices*, Gl. etun.= *Glomus etunicatum*, Gl. clar= *Glomus clarum*, Gi. Giga= *Gigaspora*

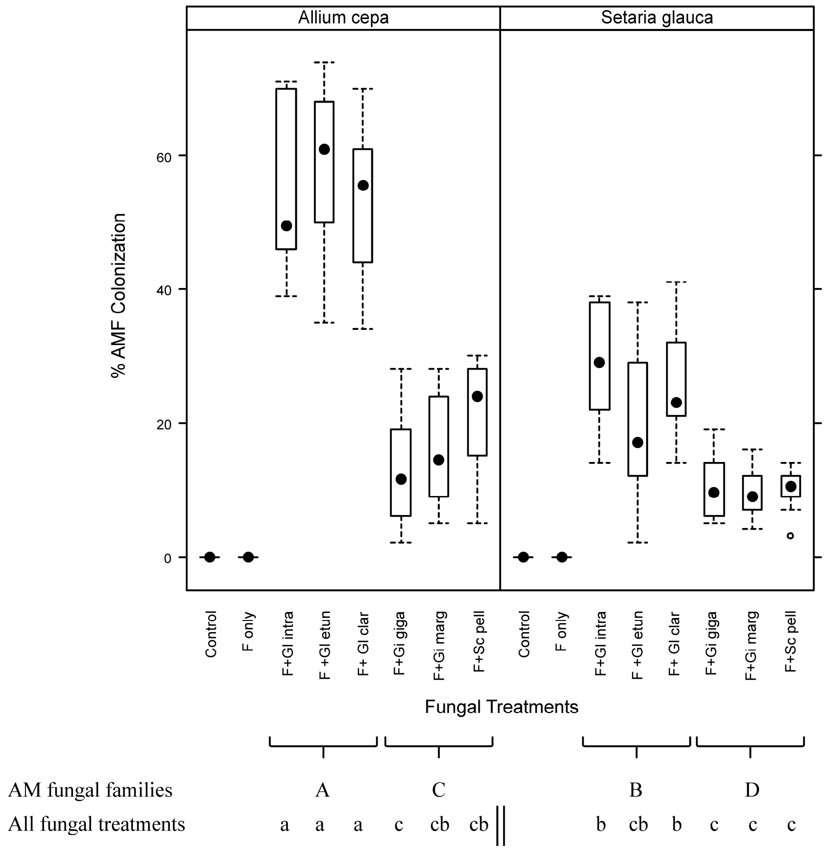
gigantea, Gi marg= *Gigaspora margarita*, Sc pell= *Scutellospora pellucida*). Closed circles represent treatment median values and open circles represent 95% outliers. Boxes enclose 50% of the data between the 25th and 75th percentile, while whiskers encompass 90% of the data.). Letters below the figure represent significant differences for Tukey tests between fungal families ($p<0.001$) and fungal additions ($p<0.05$)



AM fungal families A B B A

All fungal treatments a ab ab bc ab c c c ‖ c a c c c a a a

<u>Figure 2</u>: The effect of different fungal additions on total plant biomass of *Allium cepa* (coarse, simple roots) or *Setaria glauca* (fine, branched roots). Biomass is not compared between plants. Fungal treatments and figure symbols are as in Fig. 1.



<u>Figure 3</u>: The effect of different fungal additions on AM fungal colonization. Fungal treatments and figure symbols are as in Fig. 1.

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Title of article ("Article"): Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas

Manuscript no. (if known): JEcol-2009-0266.R1

Names of all authors in the order in which they appear in the Article: Benjamin A. Sikes, Karl Cottenie, John N. Klironomos

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