Early responses of wild plant seedlings to arbuscular mycorrhizal fungi and pathogens

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Abstract

Many plants form associations with arbuscular mycorrhizal fungi (AMF) because they profit from improved phosphorus nutrition and from protection against pathogens. Whereas mycorrhiza-induced pathogen protection is well understood in agricultural plant species, it is rarely studied in wild plants. As many pathogens infest plants in the first days after germination, mycorrhiza-induced pathogen protection may be especially important in the first few weeks of plant establishment.

Here, we investigated interacting effects of AMF and the seedling pathogen Pythium ultimum on the performance of six- to seven-week-old seedlings of six wild plant species of the family Asteraceae in a full factorial experiment.

Plant species differed in their response to AMF, the pathogen and their interactions. AMF increased and the pathogen decreased plant biomass in one and three species, respectively. Two plant species were negatively affected by AMF in the absence, but positively or not affected in the presence of the pathogen, indicating protection by AMF. This mycorrhiza-induced pathogen protection is especially surprising as we could not detect mycorrhizal structure in the roots of any of the plants.

Our results show that even seedlings without established intraradical hyphal network can profit from AMF, both in terms of growth promotion in the absence of a pathogen and pathogen protection. The function of AMF is highly species-specific, but tends to be similar for more closely related plant species, suggesting a phylogenetic component of mycorrhizal function. Further studies should test a wider range of plant species, as our study was restricted to one plant family, and investigate whether plants profit from early mycorrhizal benefits in the long term.

Zusammenfassung


In dieser Studie untersuchten wir in einem vollfaktoriellen Experiment interagierende Effekte von arbuskulärer Mykorrhiza und dem Pathogen Pythium ultimum auf das Pflanzenwachstum von Keimlingen von sechs Wildpflanzenarten, die der Familie der Asteraceae an gehören.

Die sechs Pflanzenarten unterschieden sich in ihrer Reaktion auf Mykorrhiza, dem Pathogen und deren Interaktion. Mykorrhiza hatte auf eine Pflanzenart einen positiven und der Pathogen auf drei Arten einen negativen Effekt. Zwei Arten erfuhr einen negativen Einfluss von Mykorrhiza in Abwesenheit des Pathogen und einen positiven Einfluss in Anwesenheit des Pathogens, was

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Unsere Resultate zeigen, dass sogar Keimlinge ohne etabliertem intrazellulärem Hyphennetzwerk von arbuskulärer Mykorrhiza profitieren können, sowohl mittels direktem Biomassezuwachs als auch indirekt über Mycorrhiza-induzierten Pathogenschutz. Die Funktion von Mykorrhiza variiert zwischen Pflanzenarten, ist jedoch tendenziell ähnlich für nahe verwandte Arten, was auf eine phylogenetische Komponente hindeutet. Da unsere Untersuchung sich auf die Familie der Asteraceae beschränkte, sollten weiterführende Experimente Pflanzenarten aus verschiedenen Familien miteinbeziehen und den Langzeiteffekt der mykorrhizalen Vorteile im Keimlingsstadium untersuchen.

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**Keywords:** AMF; Arbuscular mycorrhiza; Conyza; Interacting effects; Inula; Mycorrhizal function; Protection; Pathogen; Pythium ultimum; Senecio; Solidago

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**Introduction**

Plants interact with numerous other organisms, including soil microorganisms such as mycorrhizal fungi or pathogens, and understanding the nature of these interactions has become a major goal in plant ecology. Arbuscular mycorrhizal fungi (AMF) are obligate symbionts associated with the majority of plant species. In exchange for carbon (Brundrett 2009), AMF provide a wide range of functions to plants, including resource acquisition of phosphorus and other nutrients (Smith & Read 2008), improvement of water relations (Augé 2001), and protection from root-feeding nematodes and soil pathogens (Borowicz 2001; de la Pena, Rodriguez-Echeverria, van der Putten, Freitas, & Moens 2006).

The protective role of AMF is well studied in agricultural systems, especially in Solanaceae, Fabaceae and Rosaceae (Veresoglou & Rillig 2012). In these systems, AMF usually reduce disease symptoms as well as severity and increase plant survival and biomass. It is even considered a biocontrol agent for root pathogens (Borowicz 2001; Whipps 2004).

Mycorrhizal protection from pathogens can be very efficient, decreasing disease severity in plants by 30% to 42%, and is similar for a variety of pathogen species (Veresoglou & Rillig 2012). While these effects are well studied in agricultural plants much less is known on mycorrhiza-induced protection against pathogens in wild plants (Borowicz 2001). However, wild plants may differ from agricultural plants in their response to AMF and pathogens, as they were not selected to maximize yield under optimal conditions. In fact, natural selection may favor individuals with different responses to AMF and pathogens than agriculturally selected plants. One of the few wild plants investigated, Vulpia ciliata, was protected by AMF from fungal pathogens in the field (Newsham, Fitter, & Watkinson 1994; Newsham, Fitter, & Watkinson 1995a). However, as there are only few studies like these, it is currently unknown whether mycorrhiza-induced pathogen protection is equally common and important in wild as in agricultural plants.

In the context of plant invasions, it has been proposed that invasive plant species are less dependent on AMF in their introduced than in their native range, as a strong mutualistic relationship may limit the invasion success (Seifert, Bever, & Maron 2009). Moreover, introduced species were less responsive to the presence of mycorrhiza than native species (Pringle et al. 2009), but the role of AMF in plant invasions is still debated (Shah, Reshi, & Khasa 2009). Mycorrhizal effects on invasive and native species focused on nutrient up-take, but mycorrhiza-induced pathogen protection has not been addressed so far in the context of invasion.

Many plant pathogens infest seedlings in the first few days after germination when the defense mechanisms of plants are not yet fully established, and thus are the primary cause of seedling mortality (Gilbert 2002). Wild plant seedlings are not protected by fungicides as most agricultural plants and therefore may be particularly dependent on mycorrhiza-induced pathogen protection right after germination. However, studies investigating AMF function generally focused on older seedlings or on adult plants. Consequently, the role of AMF in early plant establishment is generally unclear, both in the presence and the absence of a plant pathogen.

The aim of our study was to evaluate the function of AMF in wild plant species in the critical phase after germination in the absence and presence of pathogens. Therefore, we investigated the effect of a mixture of five AMF species (+/- AMF) and the root pathogen Pythium ultimum (+/- pathogen) on six wild plant species.

**Materials and methods**

**Plant, pathogen and AMF fungi species**

We investigated the effect of AMF and a root pathogen on six plant species from four genera of the family Asteraceae: Senecio vernalis, Senecio inaequidens, Inula conyza, Conyza canadensis, Solidago virgaurea and Solidago gigantea. Three of these species, S. vernalis, I. conyza and S. virgaurea, are native to Europe whereas the others are exotics. All species form associations with AMF (Hempel et al. 2013).
Seeds of all species were obtained from Appels Wilde Samen GmbH (Darmstadt, Germany).

As pathogen treatment, we used the soil-borne Oomycota species *P. ultimum* (Pythiacae) which causes root rot and damping off (Agrios 2005). This pathogen typically infests seeds and seedlings while older plants are less sensitive (Martin & Loper 1999). It is ubiquitous and causes disease in a broad range of agricultural and ornamental plant species (Martin & Loper 1999). We used a generalist pathogen because we investigated the response of several plant species and because AMF usually induced protection against generalist pathogens (Whipps 2004). *P. ultimum* was often used in pathogen studies and its negative effects can be mitigated by AMF in agricultural plants (Kaye, Pfleger, & Stewart 1984; Starnaud, Hamel, Caron, & Fortin 1994).

All plant species selected for our study responded negatively to *P. ultimum*, based on a preliminary experiment (15–45% biomass decrease in the presence of the pathogen, data not shown).

The *P. ultimum* isolate was obtained from the Plant Pathology Laboratory of the Federal Institute of Technology in Zurich (Switzerland) and cultured on malt agar. Because plants cannot be infested directly using the malt agar culture, we inoculated autoclaved millet seeds with the pathogen and used chopped seeds to infest the plants.

As AMF treatment we used a clay-based multi-strain inoculum which was free of any other microbial species and only contained hyphae and spores of five AMF species: *Rhizobaghus intraradices* (=Glomus intraradices), *Funneliformis mosseae* (=Glomus mosseae), *F. geosporum* (=Glomus geosporum), *Claroideoglomus claroideum* (=Glomus claroideum) and *C. etunicatum* (=Glomus etunicatum) (Symiob, Lanskroun, Czech Republic). We used these five fungal species as they are very common and globally distributed (Opik, Moora, Liira, & Zobel 2006). Strains originated from Europe. We used a multi- rather than a single-strain inoculum because the ability of mycorrhizal associations to induced protection against pathogens differs among AM fungal species (Azcón-Aguilar & Barea 1996) and additive or synergistic interactions among AMF taxa can increase pathogen protection (Wehner, Antunes, Powell, Mazukatow, & Rillig 2010) and because AM fungus species occur as assemblages in both roots and soils (Oehl et al. 2003). Because we conducted a multi-species experiment and wanted to be able to compare the response of plant species to interacting effects of pathogens and AMF we did not use locally adapted plant, pathogen and AMF species for all plant habitats.

**Experimental design**

We studied interacting effects of AMF and the root pathogen *P. ultimum* on six plant species in a full factorial experiment. There were two AMF treatments, two pathogen treatments, six plant species and ten replicates resulting in a total number of 240 pots.

The experiment was conducted in two growth chambers and ran for four weeks. We surface sterilized seeds of all plant species with a 2% solution of potassium hypochlorite. Then, we sowed them into several 1-l trays filled with autoclaved seedling substrate, which was amended with 50 ml of live or sterilized AMF inoculum for the AMF and control treatment, respectively. All trays received 50 ml of a microbial wash to add the potential non-mycorrhizal microbial community of the inoculum also to control trays (Koide & Li 1989). The microbial wash was produced by mixing the AMF inoculum with water in a ratio of 1:2 and passing this blend through a 20 μm filter (Whatman 520BIII/2), thereby excluding AMF. We germinated the seeds in growth chambers (light regime: 16/8, temperature regime: 24/22).

After 10 or 17 (in Solidago species because they germinated later) days, seedlings were transplanted to 0.2-l pots containing 185 g of a 1:1 v:v mixture of autoclaved silica sand (grain size 0–4 mm) and autoclaved seedling substrate (mixture properties: pH: 6.7, P2O5: 57.5 mg/l (=0.012 g P/kg soil), 125.45 mg/l (=0.06 g N/kg), K2O: 90 mg/l (=0.035 g K/kg soil), Mg: 50 mg/l (=0.023 g Mg/kg soil), S: 50 mg S/l (=0.023 g/kg soil)).

For the AMF treatment, we mixed 10 ml of either live or sterilized AMF inoculum to the soil of each pot (van der Heijden et al. 1998; Wagg, Jansa, Stadler, Schmid, & van der Heijden 2011) and added 10 ml of a microbial wash to all of them (as described above). For the pathogen treatment, we added 5 mg of chopped millet seeds, which were either infested with *P. ultimum* or not infested to the soil. The pathogen was added at this later stage because *P. ultimum* may prevent seeds from germinating. We then planted one seedling of each species to each of the pots, using the seedlings in the +AMF and −AMF trays for the AMF treated and control pots, respectively. Then, we put the pots into 36 trays, each tray containing 6–8 pots either with or without AMF to avoid cross-contamination. We then placed these trays randomly into two growth chambers (Percival Scientific E-36L, light regime: 16/8, temperature regime: 20/19).

Within a growth chamber, trays and pots on trays were randomized twice during the course of the experiment. As the growth chambers were not large enough, we conducted the experiment in three runs under identical environmental conditions with two species per run. We watered pots every third day.

**Measurements**

We recorded longest leaf length just after planting the seedlings in the pots and every second day thereafter. Twenty-three (thirty for the *Solidago* species) days after sowing, above- and belowground seedling biomass was harvested, roots were washed and all biomass was dried at 80 °C for 3 days and weighed. Furthermore, we quantified the degree of mycorrhization at the end of the experiment. We rehydrated dry roots for 12 h, cleared them with 2.5% of KOH (15 min at
90 °C), shortly washed them in 1% HCl and stained them with 0.05% trypan blue (30 min at 90 °C). To increase the contrast, we destained the roots by keeping them in acid glycerol for 24 h. We searched the entire roots for mycorrhizal structures (hyphae, arbuscules and vesicles) under the microscope. As a control for the staining process we also collected plants in the field (two individuals of each of the species used in the experiment) and also assessed them for AMF using the same procedure as for the experimental plants.

Data analysis

We analyzed our data using analyses of covariance with species, AMF and pathogen as fixed factors. We used leaf length on the day of transplantation in the pots, i.e. when the pathogen was introduced, as co-variable to remove any variation among seedlings caused by AMF treatment prior to pathogen addition. Because plant species responded differently to AMF and pathogen as indicated by the significant species × treatment interactions (see Appendix A), we then analyzed plant species separately. The identity of growth chamber was tested, but not included in the final models as it was never significant. Also the runs were not included in the model as they were conducted under identical environmental conditions. As response variable, we used total biomass, which was highly correlated with shoot biomass, root biomass and leaf length (r = 0.98, 0.89 and 0.70, respectively). Seedlings that died during the experiment showed typical symptoms of Pythium infestation (damping-off, wizened rotten roots) and were included in the analysis with zero biomass. Seedling mortality was analyzed using a generalized linear model with the same factors as above. We do not show statistical results for the response of shoot–root ratio of single species as the statistical models were never significant. If necessary, response variables were log-transformed to meet model assumptions of the ANCOVA. All analyses were performed in R 2.14.0 (R Development Core Team 2011).

Results

Averaged over all species, we found no effect of AMF and a significant negative effect of the pathogen P. ultimum on plant biomass, leaf length (see Appendix A). Although seedling mortality was low (1 C. canadensis, 4 S. vernalis, 3 S. inaequidens), it was significantly affected by the pathogen (χ²(1) = 10.62, p = 0.001), but not by AMF (χ²(1) = 2.23, p = 0.13). However, plant species responded differently to the pathogen and interacting effects between AMF and the pathogen, indicated by the significant interactions of plant species with these factors (see Appendix A). Therefore, we present single-species analyses to better illustrate the interacting effects of the treatments (Tables 1 and 2).

The AMF treatment significantly increased biomass on average in one test species (I. conyza), but did not affect the others (Fig. 1, Tables 1 and 2). Pathogen presence significantly decreased biomass in three species (S. vernalis, S. inaequidens and C. canadensis). For one of the six plant species we found interacting effects of AMF and the pathogen on their biomass (Table 2). In S. inaequidens, AMF significantly decreased plant biomass in the absence, but increased or did not affect plants in the presence of the pathogen, indicating pathogen protection by AMF (Fig. 1B). The same pattern, though only marginally significant, was observed for S. vernalis (Fig. 1A, Table 2). The different responses among plant species to the pathogen and interacting effects of AMF and the pathogen could be mainly explained by the different responses among genera (genus × Pythium: F3,221 = 11.29, p < 0.001; genus × AMF × Pythium: F3,221 = 7.23, p < 0.001) and the similar responses within genera (Fig. 1A and B). In contrast, plant origin, i.e. European versus non-European species, did not explain differences in responses to AMF and the pathogen (origin × Pythium: F1,229 = 0.00, p = 0.95; origin × AMF × Pythium: F1,229 = 1.73, p = 0.19).

The assessment for AMF in plant roots did not show any fungal structures in both AMF and control plants. In contrast, plants collected from the field, identically treated as the experimental plants and assessed for AMF as a control for the staining process did show mycorrhizal structures.

Discussion

AMF affected plant performance without colonizing plant roots

Surprisingly, we could not detect any mycorrhizal structures in roots of both AMF and control plants, while field collected plants showed AMF structures, confirming that the protocol was appropriate. Depending on fungal isolate it may take between five days to eight weeks to detect mycorrhizal structures (Hart & Reader 2002). As we harvested our plants three to four weeks after sowing, it is likely that time for root colonization by AMF was too short. However, even though we could not observe fungal structures in the roots, AMF presence influenced plant species performance both in the presence and the absence of the pathogen. While I. conyza responded positively to AMF presence, AMF protected S. inaequidens and partly also S. vernalis from the pathogen.

There are four possible explanations for the effect of AMF without root colonization. First, the non-mycorrhizal soil community composition may have differed between pots with and without mycorrhiza treatment. However, we used sterilized soil for both AMF treatments, added sterilized AMF inoculum to the control pots and a microbial wash of the AMF inoculum to all pots. Therefore, the potential non-mycorrhizal community needed to establish in both treatments from zero and is therefore likely to be very similar in all treatments. Furthermore, because the pots received the same
amount of inoculums, which just differed in live or sterilized AMF; we think that the amount of nutrients was essentially the same in all treatments.

Second, AMF may have interacted with other soil components and thereby have changed environmental conditions for plants. The observed mycorrhiza-induced protection from pathogens may have resulted from AMF and pathogen interactions in the soil leading to weaker pathogen infection. However, this explanation is unlikely as such an effect would also have reduced infection in *C. canadensis*, which was equally negatively affected by the pathogen with and without mycorrhiza present.

Third, the plant species used may be facultative arbuscular mycorrhizal and not colonized under the specific environmental conditions in our experiment. At least *S. inaequidens* was found to be both mycorrhizal and non-mycorrhizal, but the other species were not classified as non-mycorrhizal so far (Hempel et al. 2013).

Fourth, our plants may have responded once the presence of mycorrhiza had been perceived. Plants can change their gene expression prior to direct physical contact with mycorrhiza (Harrison 2005; Parniske 2004). Using a physical barrier to separate AMF from roots of *Medicago truncatula*, Kosuta et al. (2003) demonstrated that AM fungal hyphae, but not pathogenic fungi, are perceived by roots and induce the expression of the symbiosis-related gene MtENOD11. Moreover, plants can change their defense signals upon mycorrhizal perception. Mycorrhizal plants produce salicylic acid as part of their defense program when they perceive AMF (Pozo & Azcon-Aguilar 2007). Only after cell penetration the plant activates the symbiotic program by switching from salicylic acid to higher levels of jasmonates (Pozo & Azcon-Aguilar 2007). It has also been demonstrated that AMF can affect plant chemistry and nutrition of non-host plants without infecting their roots. In the study of Fonseca, Berbura, and Daft (2001) *Brassica rapa* responded negatively to *G. etunicatum* even though roots were not colonized by AMF. Similarly, the non-host plants *Amaranthus retroflexus* (Sanders & Koide 1994) and *Ara- bis hiruta* (Grime, Hodgson, & Hunt 1988) were negatively affected by AMF without being infected. A positive response to AMF without root infection was observed for *Sinapis alba* in terms of biomass and oxidative stress (Neagoe, Jordache, Bergmann, & Kothe 2013). Since we controlled for possible nutrient and non-mycorrhizal microbial community effects of the mycorrhizal inoculum, our results suggest that our plant species responded upon mycorrhizal perception.

### Table 1

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>AMF</th>
<th>(\text{Absent} )</th>
<th>(\text{Present} )</th>
<th>(\text{Absent} )</th>
<th>(\text{Present} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Senecio inaequidens</em></td>
<td>2.40 ± 0.39</td>
<td>2.08 ± 0.18</td>
<td>3.09 ± 0.29</td>
<td>1.39 ± 0.18***</td>
<td></td>
</tr>
<tr>
<td><em>Senecio vernalis</em></td>
<td>2.38 ± 0.37</td>
<td>1.89 ± 0.23</td>
<td>2.73 ± 0.29</td>
<td>1.63 ± 0.27**</td>
<td></td>
</tr>
<tr>
<td><em>Inula conyza</em></td>
<td>1.64 ± 0.07</td>
<td>2.11 ± 0.17**</td>
<td>2.02 ± 0.16</td>
<td>1.72 ± 0.11</td>
<td></td>
</tr>
<tr>
<td><em>Conyza canadensis</em></td>
<td>0.98 ± 0.06</td>
<td>0.93 ± 0.13</td>
<td>1.13 ± 0.11</td>
<td>0.78 ± 0.07*</td>
<td></td>
</tr>
<tr>
<td><em>Solidago virgaurea</em></td>
<td>4.12 ± 0.42</td>
<td>4.71 ± 0.35</td>
<td>4.38 ± 0.43</td>
<td>4.44 ± 0.35</td>
<td></td>
</tr>
<tr>
<td><em>Solidago gigantea</em></td>
<td>3.31 ± 0.24</td>
<td>2.80 ± 0.19</td>
<td>3.21 ± 0.22</td>
<td>2.91 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>All species</td>
<td>2.47 ± 0.15</td>
<td>2.43 ± 0.14</td>
<td>2.76 ± 0.14</td>
<td>2.19 ± 0.14***</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th><em>S. inaequidens</em></th>
<th><em>S. vernalis</em></th>
<th><em>I. conyza</em></th>
<th><em>C. canadensis</em></th>
<th><em>S. virgaurea</em></th>
<th><em>S. gigantea</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf length after planting</td>
<td>1</td>
<td>35.81***</td>
<td>5.24*</td>
<td>0.45</td>
<td>5.31*</td>
<td>43.31***</td>
<td>23.90***</td>
</tr>
<tr>
<td>Mycorrhiza (AMF)</td>
<td>1</td>
<td>0.05</td>
<td>1.28</td>
<td>8.28**</td>
<td>0.25</td>
<td>0.35</td>
<td>0.10</td>
</tr>
<tr>
<td>Pathogen</td>
<td>1</td>
<td>39.80***</td>
<td>9.54**</td>
<td>2.08</td>
<td>4.69*</td>
<td>0.29</td>
<td>0.17</td>
</tr>
<tr>
<td>AMF × pathogen</td>
<td>1</td>
<td>17.08***</td>
<td>3.20</td>
<td>3.19</td>
<td>2.28</td>
<td>0.02</td>
<td>1.14</td>
</tr>
<tr>
<td>Residual df</td>
<td>35</td>
<td>33</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Adjusted (R^2)</td>
<td>0.70</td>
<td>0.29</td>
<td>0.20</td>
<td>0.18</td>
<td>0.51</td>
<td>0.35</td>
<td></td>
</tr>
</tbody>
</table>

**AMF effects differed among plant species**

The responses of plants to AMF and the pathogen differed among species. On average, AMF increased biomass of one species and did not affect the growth of the others, which confirms earlier observations of species-specific responses.
Fig. 1. Interacting effects of arbuscular mycorrhizal fungi (AMF) and the pathogen *Pythium ultimum* on total biomass of the six investigated plant species. Shown are means and standard errors.

The effect of AMF on plant performance was modulated by the pathogen in two out of six plant species. In both *Senecio* species, AMF had a negative effect in the absence, but a positive (*S. inaequidens*) or no effect (*S. vernalis*) in the presence of the pathogen, indicating mycorrhiza-induced pathogen...
protection. *C. canadensis*, which was also negatively affected by the pathogen, did not show mycorrhiza-induced protection in our study. This finding is contrary to results from agricultural studies, where mycorrhiza-induced protection is commonly observed and thought to play an important role in plant soil interactions (Borowicz 2001; Veresoglou & Rillig 2012; Whipp 2004). Because the other species were not negatively affected by the pathogen, mycorrhizal-induced protection was not required. Our study shows that pathogen protection through AMF can also occur in wild study systems. However, as not all species showed this AMF-induced protection function this may not be equally important in wild as in agricultural study systems.

Variation in responses of plants to AMF has been suggested to be caused by habitat affinity. AMF is known to be less important in ruderal plant species (Reeves, Wagner, Moorman, & Kiel 1979), although a recent plant trait analysis revealed that ruderal species were not associated with a specific mycorrhizal status (Hempen et al. 2013). In our experiments, the ruderal species (*S. inaequidens* and *S. versalis*) showed pathogen protection, but did not respond to AMF overall. In previous studies, mycorrhizal dependency was usually studied as difference in growth with and without AMF in the absence of pathogens. Our study shows the importance of investigating several functions of AMF when looking at mycorrhizal dependency of a plant.

Furthermore, differences in functional type, photosynthetic pathway, life history and morphological traits, especially root architecture and plasticity, affecting net phosphorus demands, have been suggested to cause variation in responses of plants to AMF (Koide 1991; Reinharth, Wilson, & Rinella 2012). While plant species with highly branched root systems should profit from AMF in terms of pathogen protection, species with herringbone root systems are predicted to benefit from increased phosphorus availability (Newsham, Fitter, & Watkinson 1995b; Sikes et al. 2009). Usually, annual species have a dichotomous root system while perennials have a herringbone system (Fitter, Nichols, & Harvey 1988). With only one annual species in our study, *C. canadensis*, we could not test this idea specifically, but the different response of two perennial genera (*Senecio* and *Solidago*) indicated that AMF function may still vary markedly within this group.

Even though plant species responded differently to AMF, the pathogen and their interacting effect, more closely related species responded similarly. Both *Senecio* species showed mycorrhiza-induced protection to some degree, while both *Solidago* species were unafected by AMF both in the presence and the absence of the pathogen. *I. conyza* and *C. canadensis*, the species which are less related to the others (Funk et al. 2005), showed singular responses to AMF and the pathogen. Therefore, plant relatedness seems to be more important for response to AMF and pathogen presence than a common distributional range. Our finding concurs with the observation that phylogeny can indeed explain plant responses to mycorrhiza (Reinhart et al. 2012).

### General importance of AMF in seedlings

Our study demonstrates that AMF can be beneficial for very young seedlings, contrasting earlier observations that AMF depresses seedling growth in the first few weeks after germination (Koide 1985). This observation was explained by low nutritional benefits of young seedlings by AMF, as they obtain resources from their seed when costs of carbon allocation to AMF are high. However, earlier studies only focused on growth promoting effects of AMF to plants in the absence of pathogens, neglecting the possible function of pathogen protection. In our study, those plant species in which AMF was parasitic in the absence of the pathogen were promoted or unaffected by AMF in the presence of the pathogen. This mycorrhiza-induced protection is surprising, as it is generally assumed that well-established intraradical hyphae are required to effectively protect plants from pathogens (Khaosaad, Garcia-Garrido, Steinkellner, & Vierheilig 2007; Newsham et al. 1995b; Pozo & Azcon-Aguilar 2007; Slezack, Dumas-Gaudot, Paynot, & Giannazzi 2000; Thygesen, Larsen, & Bodker 2004). Our study demonstrates that mycorrhiza-induced protection from pathogens can increase plant performance in very young seedlings even prior to root infection.

### Conclusions

In general, our study shows that the function of AMF to wild plant seedlings considerably differs among species, but that it also varies for a single plant species depending on pathogen presence. Even plant seedlings without intraradical mycorrhizal development can profit from AMF, both in terms of direct growth promotion in the absence of a pathogen and pathogen protection. Whether plants profit from this phenomenon in the long term needs further investigation. Our results further hint at a phylogenetic influence, as closely related species responded similarly to AMF while different genera responded differently, but further plant families should be investigated to ascertain this observation.

### Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.baae.2014.08.004.

References


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