Plant root architecture and the effectiveness of arbuscular mycorrhizal fungi in controlling plant pathogens

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Abstract

Arbuscular mycorrhizal fungi (AMF) may protect plants by reducing the susceptibly of roots to soil pathogens. Previous studies have suggested that differences in root architecture and AMF species may determine the degree of protection. In this experiment, we examined the effectiveness of AMF in protecting two plant species (*Andropogon gerardii, Silphium laciniatum)* with differing architecture from a common soil pathogen (*Cylindrocarpon)* in a tallgrass prairie. Our results show fibrous root systems are more susceptible to pathogen infection than simple root systems. However, high levels of AMF root colonization did not reduce pathogen infection; instead, AMF colonization and pathogen levels covaried. In addition pathogen-AMF interactions influenced root biomass (*Andropogon*) and rhizosphere enzyme activity (*Silphium*). Some of the AMF colonizing the roots were possibly less effective at providing bioprotection. As a result, plants inoculated with AMF were still susceptible to root and rhizosphere colonization by pathogens and such interactions may feedback to influence plant competition in grasslands.

Introduction

- Arbuscular mycorrhizal fungi (AMF, Glomeromycota) are known to develop symbioses with the roots of many vascular plants. This relationship enhances the nutrient (N, P) and water uptake of its host plant, and in return, the host plant provides AMF with photosynthates (i.e., sugars).

- AMF may also protect the root from soil-borne pathogens (1). However, this benefit may be a two-edged sword. Those AMF species best able to protect plants against soil pathogens may be beneficial under conditions of high pathogen abundance but detrimental when pathogens are absent because they act as a carbon sink. In addition, any benefits are largely dependent on the identity of the host plant species and composition of the AMF community (10) because certain plant-AMF combinations may be more effective at repelling pathogens than others. Further, plant susceptibility to soil pathogens may vary with root architecture. Earlier studies have shown that plants with a simple (tap) root system may be less affected by pathogens than those with complex (fibrous) root systems (1-3,10). However, these earlier experiments were undertaken in crop plants (1) or used individual AMF species (3) rather than intact AMF communities.

- In this study, we examined the effectiveness of AMF in protecting two plant species with differing architecture from a common soil pathogen in a tallgrass prairie. We compared AMF x pathogen effects in *Andropogon gerardii* (big bluestem; Poaceae) a keystone species of the tallgrass prairie with a fibrous root architecture, and *Silphium laciniatum* (compass plant; Asteraceae), a dominant perennial forb with a simple taproot system. Both species were challenged with a common soil pathogen, *Cylindrocarpon*, which is known to produce extensive necrosis of roots (6). Understanding this interaction is important because it may elucidate some of the controls over plant competition and community structure in grasslands.

We examined AMF and *Cylindrocarpon* colonization in plant roots and rhizosphere soil, plant biomass accumulation and mineral nutrition (N, P), and shifts in rhizosphere function (pH, enzyme activity) to test the following hypotheses:

1. High levels of AMF root and soil colonization reduce pathogen infection;
2. Fibrous root systems are more susceptible to pathogen infection than simple root systems;
3. Pathogen-AMF interactions influence plant health by modifying biomass accumulation and N and P levels; and,
4. Pathogen-AMF interactions operate at the root-soil interface by modifying enzyme functioning.

Methods

Experimental conditions: We initiated a factorial experiment using 2 plant species (*Andropogon*, *Silphium*) *x* 3 AMF sources (Dixon, Morton, control) *x* 2 pathogen levels (presence, absence of *Cylindrocarpon*), and five replicate pots per treatment. Seedlings of *Andropogon* and *Silphium* were propagated from locally collected seed, and grown in a 1: 1 mixture of prairie soil and coarse sand amended with whole soil inoculum from the Dixon Prairie (lower plant diversity) or Morton Grove (high diversity), or non-inoculated (control). Soil analyses (13, 14) showed that levels of plant-available NO_3 (6 ± 0.1 mg.g soil), NH_4 (4 ± 0.1), PO_4 (36 ± 0.5), and pH (7.15 ± 0.02) did not differ significantly among plant *x* AMF treatments ($P > 0.05$). After nine weeks of growth, half of the plants in each plant-AMF treatment group were challenged with a spore suspension of a pure culture of *Cylindrocarpon* species previously isolated from Dixon Prairie soils.

Harvest and analysis: After 12 weeks, plants were destructively harvested. Roots and shoots were dried and weighed, and sub-samples of the shoots were analyzed for N and P content at Kansas State University Soil Testing Laboratory. The abundance of AMF and pathogens was quantified in a) roots using sub-samples of roots stained with Trypan blue (5), and b) rhizosphere soil using sodium hexametaphosphate extraction of external AMF and pathogenic hyphae. Enzyme activity was assayed in each treatment using sub-samples of rhizosphere soil. The activity of acid phosphatase (AP), cellobiohydrolase (CBH), and N-acetyl-glucosaminidase (NAG) was estimated using the protocols detailed in Saiya-Cork et al. (2002). All data sets were tested for normality, transformed (ln x) where appropriate, and analyzed using analysis of variance (ANOVA) and post-hoc Tukey's Honestly Significant Difference (HSD) tests for significant F values.

Results

AMF and pathogen root colonization and external hyphal length

After 12 weeks of growth, 34 ± 4% root length was colonized by AMF in inoculated plants (*Andropogon* 33- 38%; *Silphium* 26- 35%; Fig. 1) compared with 0.5 ± 0.3% colonization in non-inoculated (control) plants. Fungal coils (Fig. 1) were especially abundant. Root colonization by pathogens was significantly higher in *Andropogon* than in *Silphium* (Fig. 2). In addition, the abundance of AMF was significantly correlated with pathogen abundance in the root (*Andropogon*) and in the external hyphal pool (*Andropogon*, *Silphium*; Table 1; Fig. 6).

Plant biomass accumulation and foliar N and P levels

In *Andropogon*, root biomass differed significantly among AMF treatments with the addition of pathogens (Fig. 3). Plants with Dixon AMF showed an increase in root biomass in the presence of pathogens whereas root mass in plants with Morton AMF and non-inoculated plants decreased. In *Silphium*, the presence of pathogens significantly increased shoot biomass, and foliar N and P levels (Figs. 4a, b, c respectively). However, there was no significant effect of AMF or pathogen treatment on shoot biomass, and foliar N and P in *Andropogon*, and root biomass in *Silphium*.

Rhizosphere enzyme activity

Enzyme activity in *Andropogon* differed significantly only among AMF treatments; the addition of pathogens had no significant effect on CBH and AP activity. CBH activity was significantly higher in plants with Dixon AMF than Morton AMF and non-inoculated plants (Fig. 5b). Non-inoculated plants had significantly higher AP activity than inoculated plants (Fig. 5c). Following the addition of pathogens in *Silphium*, CBH activity increased significantly in plants with Dixon AMF and Morton AMF (Fig. 5a). In addition, overall CBH activity was greater in plants with Dixon AMF than Morton AMF. However, there was no significant change in CBH activity in non-inoculated plants with pathogens. CBH activity was positively and significantly correlated with pathogen abundance on the root (*Andropogon)* and in the rhizosphere (*Silphium*; $P<0.05$). The activity of NAG did not differ significantly among plant, AMF, or pathogen treatments.

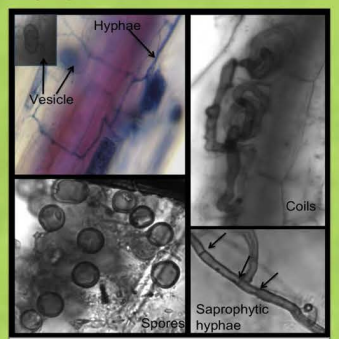

Figure 1. Vesicles (top left), spores (botton left), coils (top right), and saprophytic hyphae (bottom right). Arrows in saprophytic hyphae show septation in contrast to AMF hyphae (see coils) All images are from root samples from this experiment and show colonization by both AMF and saprophytic hyphae.

Table 1
Relationships between the abundances of AMF & saprophytic colonization, and AMF & saprophytic external hyphae in *Andropogon gerardii* and *Silphium laciniatum*. Correlation expressed as the Spearman-rank correlation coefficient.

	Root Colonization	External Hyphae
Andropogon gerardii	0.412*	0.556*
Silphium laciniatum	0.053^{ns}	0.731***

*Significant at P<0.05, ***significant at P<0.0001;ns, not significant (P>0.05); n=13 or 14 per analysis.

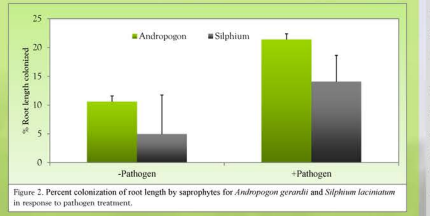

Figure 2. Percent colonization of root length by saprophytes for *Andropogon gerardii* and *Silphium laciniatum* in response to pathogen treatment.

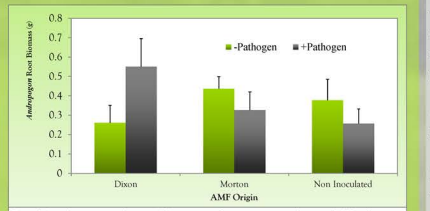

Figure 3. Root biomass accumulation in *Andropogon gerardii* in response to inoculation with AMF and with/without pathogens.

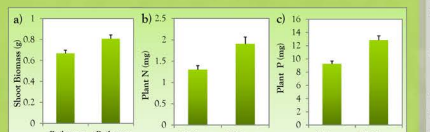

Figure 4. *Silphium* shoot biomass and foliage nutrition. a) *Silphium* shoot biomass in response to treatment with/without pathogens, b) *Silphium* nitrogen content (in mg) in response to pathogen treatment, c) *Silphium* Phosphorus content (in mg) in response to pathogen treatment.

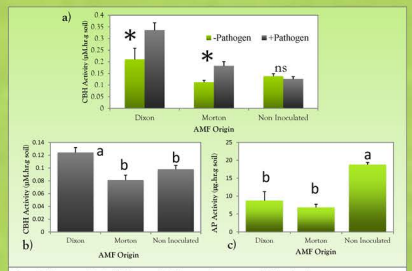

Figure 5. Enzyme activity in *Silphium* and *Andropogon* in response to AMF and pathogens. a) CBH activity in *Silphium* , b) CBH activity in *Andropogon*, c) AP activity in *Andropogon*. Columns with the same letter do not differ significantly at P<0.05. *Significant at P<0.05, ns, not significant at P>0.05.

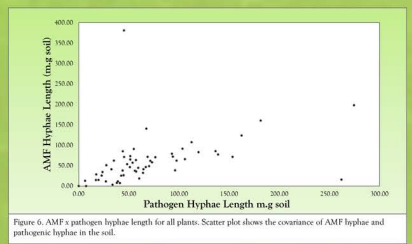

Figure 6. AMF x pathogen hyphae length for all plants. Scatter plot shows the covariance of AMF hyphae and pathogenic hyphae in the soil.

Discussion/Conclusions

Our results support the hypothesis that fibrous root systems are more susceptible to pathogen infection than simple root systems (H2). These findings are consistent with earlier studies in crop, grassland, and tropical plants (1-3,4).

Our findings also provide partial support for the hypotheses 3 and 4 in that pathogen-AMF interactions influenced root biomass (H3, *Andropogon*) and rhizosphere enzyme activity (H4, *Silphium*). The increase in *Andropogon* root biomass with Dixon AMF and pathogens suggests a local compensation mechanism whereby plants produced larger root systems (in non-infected areas) to sustain nutrient uptake. In addition, the correlation between enzyme activity and pathogen abundance in *Silphium* (external hyphal length) and *Andropogon* (root colonization) indicates that pathogens may locally influence nutrient cycling.

The experimental evidence does not, however, support the hypothesis that high levels of AMF colonization can reduce pathogen infection (H1). Instead, AMF colonization and pathogen levels covaried. Although this positive relationship was unexpected, several studies have similarly found that AMF colonization does not reduce pathogen infection (7,9,11,12). There are several ways in which this may occur:

1. The pathogen effectively competed for infection sites on the root;
2. Highly AMF-dependent plants, such an *Andropogon*, may not discriminate between AMF and soil-borne pathogenic fungi (11);
3. Those AMF species colonizing the roots, e.g., *Glomus intraradices*, were not effective bioprotectors (7);
4. Alterations in root exudation following AMF colonization may have enhanced the establishment and growth of the pathogen (9);
5. AMF activity may have reduced the activity of the pathogen without reducing the biomass (12); and,
6. AMF may have produced weak or transient bioprotection during early plant growth (7).

In addition, there may be other mechanisms-- biochemical, molecular, and ecophysiological-- that we did not test.

Overall, plants inoculated with AMF were still susceptible to root and rhizosphere colonization by pathogens, and the effects of the pathogen appeared to be largely local (versus systemic). In addition, plant species identity and, to a lesser extent, origin of the AMF inoculants, were important for determining the degree of protection or benefit a plant received from the symbiosis. Such interactions may feedback to influence plant competition in grasslands if a pathogen has a greater net negative effect on plant species with complex versus simple root architectures.

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