

1 **When do arbuscular mycorrhizal fungi protect plant roots from pathogens?**

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9 &

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24 Abstract

25 Arbuscular mycorrhizal (AM) fungi are mainly thought to facilitate phosphorus uptake in
26 plants, but they can also perform several other functions that are equally beneficial. Our
27 recent study sheds light on the factors determining one such function, enhanced plant
28 protection from root pathogens. Root infection by the fungal pathogen *Fusarium oxysporum*
29 was determined by both plant susceptibility and the ability of an AM fungal partner to
30 suppress the pathogen. The non-susceptible plant species (*Allium cepa*) had limited *F.*
31 *oxysporum* infection even without AM fungi. In contrast, the susceptible plant species
32 (*Setaria glauca*) was heavily infected and only AM fungi in the family Glomeraceae
33 limited pathogen abundance. Plant susceptibility to pathogens was likely determined by
34 contrasting root architectures between plants, with the simple rooted plant (*A. cepa*)
35 presenting fewer sites for infection. AM fungal colonization, however, was not limited in
36 the same way in part because plants with fewer, simple roots are more mycorrhizal
37 dependent. Protection only by *Glomus* species also indicates that whatever the mechanism(s)
38 of this function, it responds to AM fungal families differently. While poor at pathogen
39 protection, AM species in the family Gigasporaceae most benefited the growth of the
40 simple rooted plant species. Our research indicates that plant trait differences, such as
41 root architecture can determine how important each mycorrhizal function is to plant
42 growth but the ability to provide these functions differs among AM fungi.

43

44 TEXT

45 Arbuscular mycorrhizas (AM) represent the oldest and most widespread symbiosis with
46 land plants.¹ Most mycorrhizal research has focused on the ability of AM fungi to

47 facilitate nutrient uptake, particularly phosphorus.² Although researchers recognize that
48 AM fungi are multi-functional,³ it is not clear what factors determine which function an
49 AM fungus performs or its relative importance to the plant.⁴ Newsham et al. (1995)³
50 hypothesized that AM function is based on root architecture: plants with simple rooting
51 systems are dependent on mycorrhizas for nutrient uptake, while those with complex root
52 systems are less dependent on mycorrhizas for nutrient uptake, but are more susceptible
53 to root pathogens because of increased numbers of infections sites.³ These two functions,
54 phosphorus uptake and enhanced pathogen protection from mycorrhizas also depend on
55 the identity of the fungus. Arbuscular mycorrhizal fungi in the family Gigasporaceae are
56 more effective at enhancing plant phosphorus, while AM fungi in the Glomeraceae better
57 protect plants from root pathogens.⁵

58

59 Our results support both plant and fungal control of a common pathogen, *Fusarium*
60 *oxysporum*, and the interaction between these two factors ultimately determined the level
61 of pathogen infection and plant mycorrhizal benefit. We inoculated two plant species that
62 have contrasting root architectures with one of six AM fungal species from two families
63 (or no AM fungi). After five months of growth, plants were inoculated with *F. oxysporum*,
64 grown for another month and then harvested. All plant seeds and fungi were collected in a
65 local old field community.⁶ *Allium cepa* (garden onion) was not susceptible to *F. oxysporum*
66 likely because it has only a few adventitious roots below the main bulb that do not present
67 many sites for infection. In contrast, *Setaria glauca* (yellow foxtail) was heavily infected
68 by *F. oxysporum* and has fine roots with increased numbers of branching points and lateral
69 meristems where fungi can colonize.⁷ For the susceptible plant (*S. glauca*), AM fungal

70 species from the family Glomeraceae were effective at reducing pathogen abundance
71 while species from the Gigasporaceae were not. Forming a symbiosis with a *Glomus*
72 species resulted in *S. glauca* plants that were as large as control plants. AM fungal
73 species from the family Gigasporaceae were more beneficial to growth of the simple
74 rooted *A. cepa*, which had fewer roots to take up soil nutrients.

75

76 Reduced rooting structures may limit pathogen infection sites, but AM fungal colonization
77 was not limited in the same way and may actually alter plant root architecture. While the
78 simple rooted *A. cepa* had limited pathogen susceptibility, it had twice the AM fungal
79 colonization of the complex rooted *S. glauca*. Because the simple rooted plant has a
80 greater dependence on mycorrhizas,⁸ it likely transmits chemical signals to rapidly
81 initiate mycorrhizal formation,⁹ but then may have less control on the spread of AM fungi
82 within the root. In contrast, *S. glauca* is more susceptible to fungal pathogens and may be
83 less mycorrhizal dependent in nature¹⁰. As a result, *S. glauca* may treat all colonizing root
84 fungi as potential parasites. Colonization by AM fungi from the Glomeraceae was also
85 much greater than those in the Gigasporaceae due to major differences in fungal life
86 history strategy between these families.^{11,12} AM fungal colonization can reduce root
87 branching in plants and alter plant allocation to roots, thereby increasing mycorrhizal
88 dependence for nutrients^{13,10} and potentially reducing pathogen infection sites.
89 Mycorrhizal induced changes to plant root architecture may therefore reinforce current
90 mycorrhizal associations and alter future fungal colonization attempts.¹⁴ An important
91 next step is to test if AM fungal families (or species) alter plant root architecture in

92 different ways and the degree to which these effects depend on colonization timing and
93 the plant host.

94

95 Our study did not isolate the particular mechanism by which AM fungi control pathogens,
96 but this mechanism clearly differentiates between AM fungal families. AM fungi can control
97 pathogens through several mechanisms including direct competition for colonization sites,
98 indirect initiation of plant defensive responses or altering other rhizosphere biota.¹⁵

99 Although these AM fungal families differ in the intensity of root colonization,¹¹ percentage
100 of root length colonized by an AM fungus is a poor predictor of pathogen limitation compared
101 to family identity,^{16,12} suggesting that direct competition is unlikely. AM fungi share
102 many cell surface molecules with pathogenic fungi like *Fusarium*.¹⁷ These molecules can
103 act as signals that initiate plant production of defensive compounds such as phytoalexins,
104 phenolics, and other compounds.¹⁸ While AM fungi appear to evade these defences, AM
105 fungal species in the family Glomeraceae alone would have elicited plant responses which
106 altered future infection by *F. oxysporum*. AM fungi in the Gigasporaceae may differ more
107 from *F. oxysporum* in their chemical signals or not colonize roots sufficiently to induce a
108 sustained, system-wide plant response. In addition, many rhizosphere related microbes
109 are antagonistic to pathogenic fungi¹⁵ and may differ in their response to the different
110 AM fungal families.¹⁹ Because rhizosphere microbes also differ among plant species,
111 plant pathogen protection may be influenced by multiple ecological interactions that
112 determine the specific cases when mycorrhizal pathogen protection occurs. To distinguish
113 between these mechanisms, future experiments could test whether biochemical similarity

114 or ecological similarity (especially with other soil biota) between an AM fungus and
115 fungal pathogen can predict mycorrhizal induced pathogen protection.

116

117 Plant and fungal identity clearly affect AM fungal function and benefit, but to accurately
118 use AM fungi in agriculture and restoration^{20,21} we must clearly understand how functional
119 mechanisms differ. Different mycorrhizal functions may be based on common plant traits
120 like root architecture, but ecology, colonization timing and environment may alter the
121 specific function AM fungi provide and its importance to plants. While it may be useful
122 to establish greenhouse rules about which fungal species perform specific mycorrhizal
123 functions, predicting their role in more complex systems relies on understanding if other
124 factors will enhance or negate these effects. Most AM fungal species vary in their ability
125 to perform each function and these can be locally adapted to limiting soil nutrients.²² In
126 plants, there is also a range to which specific mycorrhizal functions may benefit plant
127 fitness, and these responses are based on both plant traits (which change throughout a
128 plant's life cycle) and the local environment.^{23,24} Given this variation, it is critical to
129 understand if AM fungi can respond to cues from the plant or the environment to identify
130 what factors limit plant growth and whether a the most effective AM fungus shows a
131 greater response.

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