

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

2

3 **Benjamin A. Sikes**

4 Department of Integrative Biology, University of Guelph, Guelph, ON, Canada N1G2W1

5

6 **Addendum to:**

7 Sikes BA, Powell JR, Rillig MC. Deciphering the relative contributions of multiple

8 functions within plant-microbe symbioses. Ecology 2010; In press

9 &

10 Sikes BA, Cottenie K, Klironomos JN. Plant and fungal identity determines pathogen

11 protection of plant roots by arbuscular mycorrhizas. J Ecol 2009; 97:1274-80

12

13 **Submitted:** 10 March 2010

14 **Accepted:**

15

16 **Key words:** Arbuscular mycorrhizal fungi, *Fusarium oxysporum*, root architecture,

17 pathogen protection, multi-functionality

18

19 **Correspondence to:**

20 Benjamin A. Sikes

21 Email: bsikes@uoguelph.ca

22

23

**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.<sup>1</sup> Most mycorrhizal research has focused on the ability of AM fungi to

47 facilitate nutrient uptake, particularly phosphorus.<sup>2</sup> Although researchers recognize that  
48 AM fungi are multi-functional,<sup>3</sup> it is not clear what factors determine which function an  
49 AM fungus performs or its relative importance to the plant.<sup>4</sup> Newsham et al. (1995)<sup>3</sup>  
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.<sup>3</sup> 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.<sup>5</sup>

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.<sup>6</sup> *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.<sup>7</sup> 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,<sup>8</sup> it likely transmits chemical signals to rapidly  
81 initiate mycorrhizal formation,<sup>9</sup> 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 nature<sup>10</sup>. 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.<sup>11,12</sup> AM fungal colonization can reduce root  
87 branching in plants and alter plant allocation to roots, thereby increasing mycorrhizal  
88 dependence for nutrients<sup>13,10</sup> 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.<sup>14</sup> 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.<sup>15</sup>

99 Although these AM fungal families differ in the intensity of root colonization,<sup>11</sup> percentage  
100 of root length colonized by an AM fungus is a poor predictor of pathogen limitation compared  
101 to family identity,<sup>16,12</sup> suggesting that direct competition is unlikely. AM fungi share  
102 many cell surface molecules with pathogenic fungi like *Fusarium*.<sup>17</sup> These molecules can  
103 act as signals that initiate plant production of defensive compounds such as phytoalexins,  
104 phenolics, and other compounds.<sup>18</sup> 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 fungi<sup>15</sup> and may differ in their response to the different  
110 AM fungal families.<sup>19</sup> 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<sup>20,21</sup> 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.<sup>22</sup> 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.<sup>23,24</sup> 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.

132

### 133 **References**

- 134 1. Brundrett MC. Coevolution of roots and mycorrhizas of land plants. *New Phytol*  
135 2002; 154:275-304.
- 136 2. Bolan NS. A critical review on the role of mycorrhizal fungi in the uptake of

- 137 phosphorus by plants. *Plant Soil* 1991; 134:189-207.
- 138 3. Newsham KK, Fitter AH, Watkinson AR. Multi-functionality and biodiversity in  
139 arbuscular mycorrhizas. *Trends Ecol Evol* 1995; 10:407-11.
- 140 4. Sikes BA, Powell JR, Rillig MC. Deciphering the relative contributions of multiple  
141 functions within plant-microbe symbioses. *Ecology* 2010; In press.
- 142 5. Maherali H, Klironomos JN. Influence of phylogeny on fungal community assembly  
143 and ecosystem functioning. *Science* 2007; 316:1746-8.
- 144 6. Klironomos JN, McCune J, Hart MM, Neville J. The influence of arbuscular mycorrhizae  
145 on the relationship between plant diversity and productivity. *Ecology Letts* 2000;  
146 3:137-41.
- 147 7. Dehne HW. Interaction between vesicular-arbuscular mycorrhizal fungi and plant-  
148 pathogens. *Phytopathology* 1982; 72:1115-9.
- 149 8. Hetrick BAD. Mycorrhizas and root architecture. *Cell Mol Life Sci* 1991; 47:355-62.
- 150 9. Harrison MJ. Signaling in the arbuscular mycorrhizal symbiosis. *Ann Rev Microbiol*  
151 2005; 59:19-42.
- 152 10. Hetrick BAD, Wilson GWT, Leslie JF. Root architecture of warm- and cool-season  
153 grasses: Relationship to mycorrhizal dependence. *Can J Bot* 1991; 69:112-8.
- 154 11. Hart MM, Reader RJ. Taxonomic basis for variation in the colonization strategy of  
155 arbuscular mycorrhizal fungi. *New Phytol* 2002; 153:335-44.
- 156 12. Powell JR et al. Phylogenetic trait conservatism and the evolution of functional trade-  
157 offs in arbuscular mycorrhizal fungi. *Proc Royal Soc B* 2009; 276:4237-5.
- 158 13. Price NS, Roncadori RW, Hussey RS. Cotton root-growth as influenced by phosphorus-  
159 nutrition and vesicular arbuscular mycorrhizas. *New Phytol* 1989; 111:61-6.

- 160 14. Kennedy P, Bruns T. Priority effects determine the outcome of ectomycorrhizal  
161 competition between two *Rhizopogon* species colonizing *Pinus muricata* seedlings.  
162 *New Phytol* 2005; 166:631-8.
- 163 15. Azcón-Aguilar C, Barea JM. Arbuscular mycorrhizas and biological control of soil-  
164 borne plant pathogens—an overview of the mechanisms involved. *Mycorrhiza* 1997;  
165 6:457-64.
- 166 16. Sikes BA, Cottenie K, Klironomos JN. Plant and fungal identity determines pathogen  
167 protection of plant roots by arbuscular mycorrhizas. *J Ecol* 2009; 97:1274-80.
- 168 17. Boller T. Chemoperception of microbial signals in plant cells. *Ann Rev Plant Biol*  
169 1995; 46:189-214.
- 170 18. Gianinazzi-Pearson V, Dumas-Gaudot E, Gollotte A, Tahiri-Alaoui A, Gianinazzi S.  
171 Cellular and molecular defence-related root responses to invasion by arbuscular  
172 mycorrhizal fungi. *New Phytol* 1996; 133:45-57.
- 173 19. Linderman RG. Role of VAM in biocontrol. *Mycorrhizae and plant health*. FL  
174 Pflieger and RG Linderman (ed). APS, St. Paul, Minnesota, USA 1994;1-25.
- 175 20. Gianinazzi, S., Schuepp, H. & Barea, J.M. *Mycorrhizal technology in agriculture:*  
176 *from genes to bioproducts*. (Birkhäuser: 2002).
- 177 21. Harris J. Soil microbial communities and restoration ecology: facilitators or followers?  
178 *Science* 2009; 325:573-4.
- 179 22. Johnson NC, Wilson GWT, Bowker MA, Wilson JA, Miller RM. Resource limitation  
180 is a driver of local adaptation in mycorrhizal symbioses. *Proc Natl Acad Sci USA*  
181 2010; 107:2093-8.
- 182 23. Klironomos JN. Variation in plant response to native and exotic arbuscular

- 183 mycorrhizal fungi. *Ecology* 2003; 84:2292-301.
- 184 24. Johnson NC, Graham JH, Smith FA. Functioning of mycorrhizal associations along  
185 the mutualism-parasitism continuum. *New Phytol* 1997; 135:575-86.