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Dual plant host effects on two arbuscular mycorrhizal fungi

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ABSTRACT

Mycorrhizal fungi may simultaneously associate with multiple plant hosts, and the implications of this for the fungi involved are not well understood. To address this question, two arbuscular mycorrhizal fungi (AMF), *Glomus claroideum* (a treatment referred to as "Glo") and *Scutellospora fulgida* (a treatment referred to as "Scut"), were grown separately in pots that each consisted of two plant compartments separated by a root-free-compartment (RFC). Fungi within each two-plant-compartment pot were exposed to either two individuals of indiangrass (*Sorghastrum nutans*), two individuals of big bluestem (*Andropogon gerardii*), or one of each. A non-inoculated treatment ("Non") was included to help gauge the potential influence of greenhouse contaminant fungi, cross-contamination, or any misidentification of non-AMF hyphae. The two host species had additive effects on the growth of AM hyphae in plant compartments of Scut, Glo, and Non pots, and in the RFCs of Scut pots. In Glo RFCs, however, they were antagonistic in their effects. Synergism between hosts in Non RFCs suggested that any potential contaminants or misidentification could not explain this result. Underyielding was not seen in shoot weight, root weight, or root length in dual host pots, and also therefore could not explain the result. Hyphal growth in the Scut treatment was evenly distributed between the RFC and plant compartments (or marginally skewed toward the RFC), while hyphal growth in the Glo treatment was skewed toward plant compartments (nearer roots). However, hyphal lengths were more highly correlated across plant compartments within a common pot in the Glo treatment, suggesting that this AMF bridged the RFC to experience the entire two-host pot as a single environment to a greater extent than Scut did. These AMF differed in how they responded to both the species composition of the two-host environment and its spatial structure; potential implications for mycorrhizal community dynamics are discussed.

1. Introduction

In natural communities, mycorrhizal fungi are often affected by multiple plant species simultaneously. Effects that plants may have on one another via shared mycorrhizal fungi have received a great deal of attention, particularly in relation to the potential redistribution of resources among plants that may occur due to hyphal linkages between plants (e.g., reviewed by Jakobsen 2004; Simard and Durall 2004). The fact that the fungi maintain these linkages and that the carbon transported via the linkages may remain within the fungal symbiote (e.g., Pfeffer et al. 2004) suggests that they are important for the fungi as well, but the effects of them on the fungi responsible have received less attention, as have interactions between plant species in their effects on mycorrhizal fungi that may occur in the absence of hyphal linkages. It is possible for example that arbuscular mycorrhizal fungi (AMF) colonizing a focal plant may be affected by inoculum from neighboring plants, or by direct or indirect influences of neighbors on the focal plant's allocation to its AMF. It has also been suggested that seasonality in plant–fungal interactions has the potential to cause fungi to benefit from occurring on multiple plant species, through extension of the fungi's growing season (Allen and Allen 1992, p. 463).

Plant community composition has been repeatedly shown to affect both AM and ectomycorrhizal fungi. The composition of the mycorrhizal fungal community on a plant's roots may be affected by the presence of neighboring plants (e.g., Johnson et al. 2003; Dickie et al. 2004; Mummey et al. 2005; Hausmann and Hawkes 2009), and this has been found even when those neighbors do not form the same type of mycorrhizas as the focal plant (Haskins and Gehring 2004). Neighbors may also influence the extent of a plant's mycorrhizal colonization (e.g., Jastrow and Miller 1993; Urcelay et al. 2003; Dickie et al. 2004; McHugh and Gehring 2006). AMF hyphal length density has been shown to increase with plant community richness (Bingham and Biondini 2009). The infectivity of mycorrhizal inoculum may be affected by the identities of plants which have previously occupied a location (e.g., Johnson et al. 1991; Dickie et al. 2006).

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Most studies that have examined the effects of host plant neighborhood on AMF have focused on the composition or aggregate growth/biomass response of an entire community of co-occurring (and presumably often competing) AMF. It is also important to examine the effects of the host plant community's makeup on separate fungi, in order to more mechanistically understand the dynamics behind AMF responses at the community level. One question of particular interest is whether multiple plant hosts interact additively, synergistically, or antagonistically in their effects on a given AM fungus. Theory suggests that non-additive effects of multiple mutualists could facilitate coexistence and diversity (Golubski 2002) and/or affect the relative fitnesses of generous vs. exploitative mutualistic partners (Golubski 2007). Synergistic effects of multiple AMF on a shared host plant have been observed (Jansa et al. 2008), and the importance of synergistic effects of multiple mutualists over long timescales has recently been shown for ant-acacia mutualisms (Palmer et al. 2010).

Here, I examine the growth response of arbuscular mycorrhizal fungi (AMF) to pairs of conspecific or heterospecific hosts separated by small root-free compartments (RFCs), in order to determine whether the two host species are additive, synergistic, or antagonistic in their effects on the fungi. Two AMF of differing genera, *Glomus clairoideum* Schenck & Smith (which I will refer to as "Glo" for conciseness) and *Scutellospora fulgida* Koske & Walker (referred to as "Scut"), were used, as well as a non-inoculated treatment (referred to as "Non"). Two C4 prairie grasses, indiangrass (*Sorghastrum nutans* (L.) Nash) and big bluestem (*Andropogon gerardii* Vitman), were used as the host plant species. Hyphal length per gram dry soil was measured as the indicator of AMF growth. I also examine whether any of three plant parameters (shoot weight, root weight, or root length) predict fungal growth in each combination of host and fungal species, and/or are able to explain the response of a fungus to the combination of the two plant hosts. The effects of the fungi on these plant parameters as well as three parameters that are constructs of them: whole plant dry weight, root:shoot ratio, and specific root length (SRL; m root per g dry weight), are also shown.

2. Methods

2.1. Study species

Big bluestem and indiangrass were chosen as hosts in large part because of their similarities (both being large C4 prairie grasses). Synergism or antagonism would be indicated by an increase or depression of fungal growth when both plants are present relative to what would be expected based on fungal growth with each plant alone. If a fungus' growth on one species differed greatly from its growth on another, synergism or antagonism between hosts might be more difficult to detect and the results would be more sensitive to discrepancies between what the fungus perceives the relative abundances of the two hosts to be vs. what relative abundances are assigned in the analyses. Acting in opposition to that concern is the reasoning that greater differences between plant species should provide greater opportunities for those plants to interact synergistically or antagonistically (i.e., not be functionally redundant) in their effects on fungi. Fungal species were chosen to represent two families (Gigasporaceae (*S. fulgida*) and Glomeraceae (*G. clairoideum*)) that tend, in general, to differ in their relative amounts of internal vs. external mycelium (e.g., Hart and Reader 2002b) as well as relative dependence on different structures as inoculum (Klironomos and Hart 2002). This was done in order to attempt to examine the responses of two AMF species that are as different as possible.

2.2. Setup

Pots were constructed from 4 in. schedule 40 PVC. They consisted of two plant compartments 33 cm tall, each of which held approximately 800 ml of medium, located on opposite sides of a root-free-compartment (RFC). Each plant compartment was separated from the RFC by 20 μm nylon mesh, through which hyphae, water, and nutrients could pass but roots could not. The RFC ranged from 0.5 cm wide at their narrowest point to 2 cm at their widest. Pots were assigned to one of three inoculum treatments: Glo, Scut, or Non (as outlined above). In each of these treatments, there were six replicate pots in which an individual of big bluestem would be grown in each of the two plant compartments, six pots in which an individual of indiangrass would be grown in each plant compartment, and 6 pots in which a big bluestem individual would be grown in one plant compartment and an indiangrass individual in the other. The former two treatments are referred to as "single host" treatments while the latter is referred to as the "dual host" treatment. Hosts were quantified by number of individuals, so the ratio of hosts in the dual host treatment was 1:1. Supplementary Fig. S1 illustrates the pots used. Four pots were excluded from analyses because one plant had died by the end of the experiment; these represented one replicate from each of the following treatments: single host big bluestem with Scut inoculum, single host indiangrass with Non inoculum, dual host with Non inoculum, and dual host with Glo inoculum.

Plant seeds were purchased from the Prairie Moon Seed Nursery (Winona, MN; www.prairiemoon.com). They were surface sterilized for 5 min in 5% bleach, then rinsed and stratified in wet sand in a refrigerator for 6 weeks. Fungal inocula were obtained from James Bever, and had been grown from isolates originally collected from an Indiana prairie. Each inoculum was bulked up on sudangrass (*Sorghum bicolor* (L.) Moench subsp. *drummondii* (Steud.) de Wet) for 4 months in a growth chamber, with a 2-week period at the end during which pots (with plants still present) a were allowed to dry to encourage sporulation. A homogenized mixture of plant roots and medium (with roots cut to approximately 1 cm segments) was used as experimental inoculum. The resulting material was diluted 1:10 with pasteurized medium to produce the inoculum for the experiment. Pasteurized medium consisted of 9 parts autoclaved sand to 1 part prairie soil which had been collected from Fermi National Accelerator Laboratory, passed through a 1 mm sieve, and autoclaved twice at 100 °C for 1 h with 24 h between the two autoclaving steps. All RFCs, as well as plant compartments of Non pots were filled with only pasteurized medium; plant compartments in Glo or Scut pots were inoculated with 250 ml of the appropriate inoculum, located as a band approximately 3/4 of the way to the top of the compartment. This corresponded to approximately 1/6 of the volume of each compartment after taking into account the amount by which the medium settled when watered for the first time. Plant seeds were added to the top of the medium in each plant compartment, and covered with autoclaved gravel.

The experiment was planted on June 25, 2004. Plants were thinned to one per pot on July 16, and grown in a greenhouse through December 6. The greenhouse was not air-conditioned, and windows were whitewashed to control temperatures, which reduced the amount of light available to the plants. Plants were provided with some supplemental (fluorescent) lighting to partially compensate for this. Each plant compartment was given 100 ml of deionized water daily, except for twice weekly when they were instead given 100 ml of nutrient solution (one teaspoon of 20-20-20 fertilizer (Peters Professional, Scotts Miracle-Gro Company, Marysville, OH) diluted in 4 gallons of deionized water). Fertilization began in the 3rd week of plant growth after thinning; it was originally hoped that the changing conditions that might be generated through the course of the experiment by depletion of a