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Short communication

Glomalin production by an arbuscular mycorrhizal fungus: a mechanism of habitat modification?

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Abstract

We report on a novel mechanism by which arbuscular mycorrhizal fungi may be able to modify a sub-optimal growth environment to their advantage by secreting the glycoprotein glomalin, which is highly correlated with soil aggregate water stability. Here we used glass beads of different sizes to simulate different hyphal growing space conditions. With small beads (<106 μm; simulating a non-aggregated soil) fungal hyphal length was reduced over 80% compared to large beads (710–1180 μm; simulating an aggregated soil). Conversely, glomalin yield (immunoreactive protein) was more than seven times higher in the small bead growing space. The results challenge our thinking about soil aggregation as a consequence of general biological activity in soil, and uncover a new aspect of the biology of these ecologically important soil fungi.

Keywords: Arbuscular mycorrhiza; Glomalin; Soil aggregation; Glass beads; In vitro culture

A current paradigm in ecology focuses on the importance of responses of organisms to their environment, and much less emphasis is placed on how biota may modify their environment to render it more favorable for their growth (Jones et al., 1997). There is general agreement that soil structure, formed by a complex hierarchical arrangement of aggregates, is a habitat feature that is greatly influenced by the activity of soil biota (Miller and Jastrow, 2000). However, it is tempting to conceptualize soil aggregation, due to its complexity, as merely a by-product of the numerous activities of biota, rather than as a process that may involve regulation by or specific feedback responses of members of the soil community. Soil organic matter plays a major role in aggregation, and organic matter and its modification are necessary results of soil biota activities in general (Paul and Clark, 1996). With the discovery (Wright and Upadhyaya, 1996) of a specific substance, called glomalin, involved in soil aggregation, this view could be challenged. Glomalin is a glycoprotein produced copiously by arbuscular mycorrhizal fungi (AM) fungi (Rillig et al., 2001), and its concentration in soil is strongly positively correlated with the water-stability of soil aggregates (Wright and Upadhyaya, 1998; Rillig et al., 1999a). Not many details on the molecular properties of glomalin are known, but the protein contains iron (Rillig et al., 2001), appears to have N-linked oligosaccharides (Wright et al., 1998), and is insoluble and possibly hydrophobic in its native state (Wright and Upadhyaya, 1996; Wright et al., 1996).

Soil structure is important for facilitating water infiltration, biogeochemical cycling processes, resistance against erosional soil loss, and soil carbon storage (Oades, 1984; Elliott and Coleman, 1988; Hartge and Stewart, 1995; Jastrow and Miller, 1997). Functions of glomalin production at the ecosystem scale are hence apparent. However, producing this protein and secreting it into the environment constitutes a large carbon (and nitrogen) cost for the obligately biotrophic AM fungus, and the functional importance of glomalin production to the fungus is so far unknown. Here, we hypothesized that the fungus could benefit directly from the production of glomalin by improvement of its physical growing space, i.e. that the production of glomalin contributes to the physical engineering of soils sensu Jones et al. (1997).

In order to test if hyphal growth and glomalin production are responsive to physical growing space, we need to compare aggregated and non-aggregated soil. It is inherently difficult to compare function of AM fungal hyphae in aggregated and non-aggregated soil, since this very difference arises from dissimilarity in soil organic matter content, communities of biota and a host of other

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Table 1
Responses of roots, mycorrhizae, hyphae, and glomalin to AMF hyphae growing in small or large glass beads. Glomalin is reported as Bradford-detectable and immunoreactive (IR) protein. Means ($n = 4$) are shown with standard errors of the mean (brackets). *$P < 0.05$ (Mann–Whitney U test)

	Small beads	Large beads
Root length (10^{-3} m)	434 (133)	539 (124)
Root weight (10^{-3} g)	29.2 (9.3)	36.7 (7.3)
AMF root colonization (%)	70.8 (5.3)	76.5 (1.6)
Bradford glomalin (10^{-6} g)	24.1 (11.2)	6.8 (0.6)*
IR glomalin (10^{-6} g)	1.08 (0.23)	0.15 (0.02)*
AMF hyphal length (m)	0.82 (0.60)	4.97 (1.56)*

parameters. These features would hence represent major confounding factors. In this experiment, we therefore simulated the aggregation status of soil by using different size glass beads (Parr et al., 1963). We hypothesized that yield of AMF hyphae would be greater in a medium simulating aggregated soil (large glass beads), reflecting increased hyphal growth, compared to a medium simulating non-aggregated soil (small beads). We further hypothesized that yield of glomalin would be greater in the simulated non-aggregated vs. aggregated soil.

Transformed carrot (*Daucus carota* L. DC1) roots colonized with the AM fungus *Glomus intraradices* were grown in vitro in split-compartment Petri dishes ($n = 4$) according to St-Arnaud et al. (1996). Roots were restricted (by pruning) to the root compartment filled with M Phytagel growth medium with sucrose (St-Arnaud et al., 1996), while AM fungal hyphae could proliferate in a hyphal compartment (where roots had no access) without sucrose. Glass beads (Sigma; small: <106 μm; large 710–1180 μm) were acid-washed (48 h), and then rinsed extensively with deionized water until wash water had the same pH as deionized water. Glass beads were autoclaved, oven-dried, and added (20 ml per plate; plates were gently tapped to distribute glass beads in the compartment) to the hyphal compartment under sterile conditions after roots had grown in the root compartment for 30 d. Average calculated total pore space was 56 and 57% in the large and fine beads, respectively, assuming open packing. Five ml of liquid M medium (no sucrose, no gelling agent) were added to the glass beads by pipetting (1 ml pipette) in a zigzag pattern across the beads to coat them as uniformly as possible. Uniformity of liquid distribution was assessed visually in the small beads, and no obvious patchiness was observed. There was minimal bottom ponding in the large beads, visible as a thin film. Plates were incubated at 25 °C for another 30 d and then destructively harvested. We microscopically examined hyphal growth prior to destructively harvesting the compartments. Hyphae were visible on the top and bottom surface of the bead mass, but we could not examine the distribution of hyphae in the center portion of the bead mass.

Roots were extracted by solubilizing the Phytagel growth medium (Doner and Becard, 1991), rinsed, dried (100 °C), and weighed. Root lengths were measured using the WinRhizo system (Regent Instruments, Quebec, Canada). In the hyphal compartment, glomalin was extracted from a 10 g bead sub-sample with 8.0 ml nanopure water by autoclaving for 30 min at 121 °C. Glomalin was quantified by ELISA using monoclonal antibody MAb32b11 (Wright and Upadhyaya, 1996) and also using a Bradford total protein assay (Wright and Upadhyaya, 1996). While the Bradford assay is not specific to glomalin (as opposed to the ELISA), the extraction procedure (autoclaving) eliminates any proteins that are not heat stable. We showed by SDS–PAGE banding patterns (Rillig et al., 2001) that glomalin crude extract (as measured in the Bradford assay) does not contain any significant amounts of protein other than glomalin. We do not know what controls immunoreactivity of the protein at this point, and hence we report both fractions of glomalin. Hyphae were extracted from glass beads and quantified with an aqueous membrane-filtration method (Rillig et al., 1999b). Using repeated extractions of extracted beads, hyphal extraction efficiency was not found to be different between large (98%) and small (97%) bead treatments. Owing to non-normal distribution of data, we used Mann–Whitney U tests to test for differences among means.

Root length (measured in the root compartment) was not significantly different ($P > 0.05$) between the small and large beads (Table 1). Root weight was also not significantly different (Table 1). Likewise, percent root colonization with mycorrhizal structures was not significantly different between small and large beads (Table 1). Total Bradford-determined protein concentration (per compartment) was significantly lower in the large beads than in the small beads (Table 1). This was also the case for the immunoreactive glomalin. Hyphal length (per compartment) was significantly higher in the large than in the small beads (Table 1). When normalized for root growth, yield of hyphae was 6.8 times higher in the large glass than in the small beads; conversely, immunoreactive glomalin yield expressed on a hyphal length basis was significantly lower in the large than in the small beads (Fig. 1).

At the cost of having used an artificial system, we could exclude the effects of other organisms and processes potentially involved in aggregation. Our results indicate that under less favorable growing conditions, representing a non-aggregated soil, increased amounts of glomalin can be secreted into the growth medium, despite a much smaller hyphal length. The results support our hypotheses and clearly show that AM fungi can respond to their growing space. Furthermore, this response is highly suggestive of a reaction that would, in the field, lead to increased soil aggregation, due to the strong and positive correlation of glomalin concentration with soil aggregate water stability across a variety of soils ($r^2 = 0.71$, $n = 31$; Wright and Anderson, 2000) and within a soil ($r^2 = 0.78$, $n = 54$; Wright et al., 1999).

AM fungi are an evolutionarily ancient group of symbionts, having possibly co-evolved with land plants for 460 million years (Redecker et al., 2000). This may have

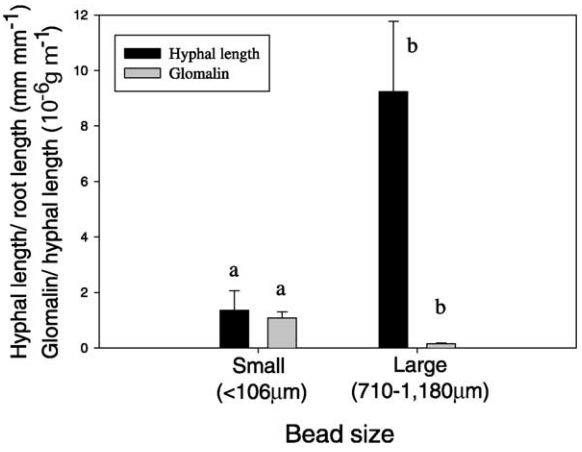

Fig. 1. Hyphal length (normalized for root length) and glomalin yield (per hyphal length) of the AF fungus *G. intraradices* when grown in vitro in small or large glass beads. Means ($n = 4$) were compared with Mann–Whitney U tests (significance accepted at $P < 0.05$). Error bars are standard errors of the mean.

exposed AM fungi to selection pressure leading to the development of mechanisms that enhance their soil growing space (extended phenotype engineering sensu Jones et al., 1997). The mechanism and potential regulatory pathways of this response are as yet unknown, and could include a generalized stress response of the fungus, or a more specific response to sub-optimal soil aggregation. An alternative explanation for the results we observed could be that as the fungus allocates resources to rapid growth (large beads), less C is allocated to production of secreted proteins, such as glomalin. If this is a specific response to the physical growing space, then the nature of the trigger leading to the observed response is unknown. Clearly, along with different particle size many changes to the hyphal growth environment arise, such as changes in water potential, gas exchange, and penetration resistance. It would be interesting to further examine if any of these environmental factors are perceived by the fungus and can potentially trigger a change in glomalin production.

Other studies have attempted to measure effects of different growing space on AM fungi by using different-sized particles (Millner and Kitt, 1992; Gaur and Adholeya, 2000). However, these studies have not taken into account different chemical properties of the different-sized particles sizes used. Our study is one of the first to specifically demonstrate the effect of physical growing space as a factor in AM mycelia's growth. The results suggests that regulatory responses and mechanisms at the organismal level should be a focus of further research on soil structure, and could complement more process-functional or physico-chemical fractionation approaches.

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