SHORT COMMUNICATION

Glomalin production and accumulation in soilless pot cultures

Kristine A. Nichols

USDA-ARS, Northern Great Plains Research Laboratory, P.O. Box 459, 1701 10th Ave SW Mandan, ND 58554 USA (e-mail: Kristine.Nichols@ars.usda.gov). Received 6 May 2009, accepted 17 August 2010.

Nichols, K. A. 2010. Glomalin production and accumulation in soilless pot cultures. Can. J. Soil Sci. 90: 567–570. This study was designed to determine how glomalin production relates to accumulation in three sequential greenhouse culturing periods using a soilless potting medium. The data indicated that this stable glycoprotein was washed out the pot bottom, and did not accumulate in the hyphal chamber.

Key words: Arbuscular mycorrhizal fungi, Bradford reactive soil protein, glomalin, hyphal chamber, soilless pot cultures, root chamber


Mots clés: Mycorhize à arbuscules, protéine du sol sensible au réactif de Bradford, glomalone, chambre hyphale, culture en pot sans sol, compartiment des racines

Glomalin research to date has relied on quantification using the Bradford total protein assay [i.e., Bradford-reactive soil protein (BRSP)] or ELISA with an antigliomalain antibody (i.e., glomalin-related soil protein) (Nichols and Wright 2004; Rillig 2004; Treseder and Turner 2007). These measurements are made on protein crudely extracted from soil samples, in-growth cores, and pot culture experiments. Prolonged and repeated high temperature (121°C) incubations in an alkaline salt solution are used to extract a mixture of both freshly produced and residual glomalin which may have accumulated over decades to centuries (Wright and Upadhyaya 1999; Nichols and Wright 2004; Rillig 2004; Treseder and Turner 2007).

Glomalin values have been compared with water-stable aggregation measurements or particular agronomic practices (Wright and Upadhyaya 1999; Rillig 2004; Treseder and Turner 2007). These studies are limited to corollary relationships between glomalin and some soil parameter or management scenario. To begin determining a direct relationship between glomalin and soil, a methodology for separating freshly produced glomalin from background glomalin levels accumulating over time is needed. Early glomalin research indicated modifications in extraction protocol – a single 30-min high temperature extraction with 20 mM sodium citrate, pH 7.0 [easily-extractable glomalin (EEG)] versus repeated 60-min high temperature extractions with 50 mM sodium citrate, pH 8.0 (total glomalin – TG) may separate freshly produced (EEG) glomalin from residual (TG) glomalin (Nichols and Wright 2004; Treseder and Turner 2007). However, more recent studies showed glomalin moving into and out of the EEG and TG pools during soil incubations even in the absence of growing arbuscular mycorrhizal fungi, raising questions about the accuracy of extraction-based separations (Treseder and Turner 2007). In-growth cores filled with soilless media were used to study glomalin production in field settings (Lovelock et al. 2004; Nichols and Wright 2004; Treseder and Turner 2007). However, it was difficult to keep these cores from getting contaminated by the surrounding soil and to extract enough glomalin for accurate quantification (S. F. Wright, personal communication).

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Abbreviations: AM, arbuscular mycorrhizal; BRSP, Bradford-reactive soil protein; EEG, easily-extractable glomalin; TG, total glomalin

Understanding the ecophysiology of glomalin will solidify our knowledge of how and why arbuscular mycorrhizal fungi produce glomalin and how glomalin functions in the rhizosphere. To make these measurements, methodology needs to be developed to accurately separate freshly produced and residual glomalin. The hypothesis for this study was that glomalin accumulation would be proportional to glomalin production.

A soilless pot culturing system designed by Millner and Kitt (1992) was modified to use a sand:crushed coal (1:1, vol/vol) mix as the potting medium and separate roots from fungal hyphae with using a seamless, 38-μm nylon mesh bag penetrable by hyphae but not plant roots (Nichols and Wright 2004). The potting medium was pre-extracted to remove background glomalin levels.

Three corn (Zea mays) seedlings (at the two-leaf stage) inoculated with spores and hyphae of either Gigaspora rosea (Nicholson and Schenck) or Glomus etunicatum (Becker and Gerdemann) were planted in the root chamber. Pots were placed randomly on a greenhouse bench under controlled temperature and lighting conditions and watered by drip irrigation four times daily with a reduced phosphorus (P) (40 μM for the first 4 wk and 20 μM for the remaining 10 wk) half-strength Hoagland’s solution until water flowed from the pot bottom (Millner and Kitt 1992). Drain holes on the sides of the pots were covered with tape and those on the bottom were covered with horticultural mesh.

Cultures were maintained for 14 wk, after which a third of the pots were harvested while the root chambers in the remaining pots were removed and replaced for another 14-wk culturing period. A total of three culturing periods were conducted for each mycorrhizal species. Culture periods for G. etunicatum were from Jan. 14 to Apr. 22, Apr. 22 to Jul. 29 and Jul. 29 to Nov. 04 2002 and for G. rosea from May 21 to Aug. 27, Aug. 27 to Dec. 04 and Dec. 08 2002 to Mar. 15 2003. The root chambers were removed to measure glomalin production during each culturing period and replaced with a new root chamber containing uninoculated seeds. Seeds were uninoculated to maintain inoculum density at original levels. If a corn root penetrated the hyphal chamber, these pots were removed from the study.

Glomalin extractions were performed on the root and hyphal chambers separately using the TG extraction protocol, and glomalin was quantified by the Bradford total protein assay (Lovelock et al. 2004; Treseder and Turner, 2007; Wright and Upadhyaya 1999). A BRSP ratio was calculated for each chamber and culturing period by dividing the BRSP value obtained in that culturing period by the BRSP average from the previous period. This calculation removed problems stemming from background glomalin present in the potting media even after pre-extraction and any co-extracted proteins falsely measured by the Bradford total protein assay. Since the BRSP in the root chamber was most likely similar for each pot at the beginning of the culturing period, the BRSP measured in this chamber at the end of each culturing period would indicate glomalin production during that period. Because BRSP values in the hyphal chamber included glomalin present in this chamber from both the previous culturing period(s) and the current culturing period, these values were used as glomalin accumulation. Different fungal species have different glomalin production levels requiring each species to be analyzed separately.

Statistical analysis was performed using SAS with the means of the BRSP ratios among culturing periods compared by analysis of variance (ANOVA) (Proc Mixed). Correlation coefficients (Proc Corr) were calculated between the production and accumulation BRSP ratios to identify the relationships over sequential culturing (SAS Institute, Inc. 2002).

Previous research has shown glomalin resists decomposition, especially in short-term studies (Treseder and Turner 2007). Therefore, with each additional culturing period, glomalin accumulation in the hyphal chamber was expected to increase proportionally to glomalin production over the current and previous culturing periods. Also, the accumulation ratios should never be statistically less than 1. Even if production values between periods were maintained or declined compared with the previous culturing period, the amount of BRSP present in the hyphal chamber should be at least equal to the previous culturing period unless glomalin was lost from the hyphal chamber.

Bradford-reactive soil protein production in the hyphal chamber increased by over twofold between the first and second culturing periods for G. etunicatum and then declined significantly from period 2 to period 3 (Table 1). For G. rosea, production levels were maintained from period 1 to period 2 but again significantly declined from period 2 to period 3 (Table 1). Accumulation followed a similar pattern for the first two culturing periods of both the G. etunicatum and G. rosea cultures (Table 1). Although significant, the increase in BRSP accumulation by the second culturing period for G. etunicatum was only about 1.4 times. Since accumulation is a measurement of glomalin remaining from the previous culturing period and produced during the current period, these values actually should be a combination of all previous production levels or should have quadrupled for G. etunicatum and doubled for G. rosea. Because little glomalin was produced during the third culturing period for either fungal species, accumulation levels were maintained as expected. The correlation coefficients between production and accumulation were less than 0.5[|r| = 0.4846 (Prob > |r| = 0.16) and |r| = 0.4506 (Prob > |r| = 0.08) for G. etunicatum and G. rosea, respectively] showing a weak relationship and, in the case of G. rosea, a negative relationship.

Two aspects of this study may have impacted photosynthetic activity and C allocation, which, as a consequence, probably influenced glomalin production. First, although additional lighting was provided in the
greenhouse, ambient irradiance due to seasonal differences visibly affected plant growth and photosynthesis. This was particularly apparent during the third culturing period, which was conducted over the fall. Second, removing the root chamber from the pot would break the connection between fungus and plant requiring C resources to be allocated to new root colonization and reforming the connection between the root and hyphal chambers rather than glomalin production. Studies reviewed by Treseder and Turner (2007) support this link between C from the plant and glomalin production. However, this information relates only to glomalin production and not accumulation.

To determine why BRSP accumulation ratios did not double or quadruple throughout this study, the water running out of the pot bottom was examined. The automatic watering system used in this study was originally designed to prevent a porous, unreactive potting medium, such as sand, from drying out (Millner and Kitt 1992). However, watering the pots until water flowed from the bottom four times a day may have had an unintended consequence, namely washing glomalin out of the hyphal chamber. Although glomalin is highly insoluble at room temperature (Wright and Upadhyaya 1999; Nichols and Wright 2004), BRSP has been measured to depths of 1.4 m and in the form of five US rivers, primarily due to glomalin movement through and from soil on water films (Harner et al. 2004). In addition, in a pot culture experiment similar to this one with the same sand:crushed coal potting mix and watering system, brownish scum was found on the bottom and sides of the plastic containers used to catch the water dripping from the pots. Some of this material was collected and found to be highly immunoreactive with BRSP concentrations of 4.47 and 6.33 mg BRSP g⁻¹ of material collected. Also, a foamy material, noted when emptying the waste water containers and collected on a 53-µm mesh screen, was highly immuno-reactive. Finally, immuno-fluorescence assays, performed on the horticultural mesh placed over the bottom drain holes in this study, found highly immuno-reactive material on and around the holes in the mesh (Fig. 1). These results held up the assumption of glomalin being washed from the pot making the accumulation data questionable.

Although the hypothesis that accumulation would be proportional to production was inconclusive, this study did have some important conclusions. The data supported the interaction between plant and fungal C allocation and glomalin production and showed the importance in examining the waste water flowing from the pot culture for glomalin. Therefore, any future pot culture and field studies relating glomalin concentration to soil parameters, such as aggregation, and agronomic practices, should consider measuring plant biomass, root colonization, or hyphal length and glomalin concentration in waste water or runoff.

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**Table 1. The production and accumulation ratios for Bradford Reactive Soil Protein (BRSP) measured in soilless pot cultures Glioma etunicatum and Gigaspora rosea. Ratios were calculated by comparing BRSP values from one culture period to the average values from the previous period.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Culturing periods</th>
<th>Glomalin production ratio (BRSP in the root chamber)</th>
<th>Glomalin accumulation ratio (BRSP in the hyphal chamber)</th>
<th>Production to accumulation ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glioma etunicatum</td>
<td>1</td>
<td>1.00±0.03b</td>
<td>1.00±0.13b</td>
<td>1.10±0.17ab</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.16±0.29a</td>
<td>1.39±0.11a</td>
<td>1.61±0.35a</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.36±0.1c</td>
<td>1.00±0.06b</td>
<td>0.36±0.01b</td>
</tr>
<tr>
<td>Glioma rosea</td>
<td>1</td>
<td>1.00±0.31A</td>
<td>1.00±0.33A</td>
<td>1.82±0.83A</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.88±0.06A</td>
<td>1.00±0.04A</td>
<td>0.90±0.10A</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.39±0.03A</td>
<td>1.29±0.14A</td>
<td>0.34±0.07A</td>
</tr>
</tbody>
</table>

α–e, A, B Values in a column followed by different letters indicate significant differences at Prob > F < 0.1. Lower-case letters are for Glioma etunicatum; upper-case letter are for Glioma rosea cultures.
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**Wright, S. F. and Upadhyaya, A. 1999.** Quantification of arbuscular mycorrhizal fungi activity by the glomalin concentration on hyphal traps. Mycorrhiza 8: 283–285.