On-farm production of inoculum of indigenous arbuscular mycorrhizal fungi and assessment of diluents of compost for inoculum production

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1. Introduction

Arbuscular mycorrhizal [AM] fungi are native to agricultural soils and form a mutualistic symbiosis with the majority of crop plants. Among the benefits to the plant ascribed to the symbiosis are enhanced: uptake of immobile nutrients, water relations, and disease resistance (Smith and Read, 2008). These benefits make utilization of the symbiosis attractive to sustainable agricultural systems that are designed to minimize synthetic inputs of fertilizer and pesticides, or in the case of organic agriculture, eliminate them.

Inocula of AM fungi are available commercially in a variety of forms ranging from high concentrations of AM fungus propagules in carrier materials to potting media containing inoculum at low concentrations. Effective AM fungus inoculum may also be grown on-the-farm using a variety of methods. These techniques were pioneered in Columbia (Dodd et al., 1990; Sieverding, 1991) and India (Gaur, 1997; Gaur and Adholeya, 2002), but we have recently developed a method suitable for temperate climates (Douds et al., 2006). This method entails mixing compost with vermiculite to decrease the effective concentration of available nutrients in the compost, notably of P (Douds et al., 2008a). High levels of P are known to inhibit colonization of roots by AM fungi (Hepper, 1983). Bahiagrass (Paspalum notatum Flugge) seedlings, colonized by specific AM fungi, are then transplanted into bags containing the compost and vermiculite mixture. The AM fungi proliferate in the media as the bahiagrass grows throughout the summer, and the bags are weeded and watered as needed. The bahiagrass is then frost killed. The AM fungi over winter in the media and the inoculum is ready for use the following spring. Inoculum produced in this fashion has increased the yield of potatoes (Douds et al., 2007) and strawberries (Douds et al., 2008b).

Adoption of on-farm production of AM fungus inoculum in compost and vermiculite mixtures is hampered by two factors. First, the lack of commercially available seedlings already colonized by selected AM fungi means there is no starter inoculum of the form outlined in the procedure above. Second, the possibility of asbestos contamination of certain sources of vermiculite may cause hesitation in adoption of the method (US EPA, 2008). Experiments were conducted to find an alternate starter inoculum to allow farmers to propagate the AM fungi indigenous to their farms and test the use of alternatives to vermiculite for dilution of the compost.

2. Methods

2.1. Propagation of indigenous AM fungi

Two methods were tested for the propagation of indigenous AM fungi. The first entailed adding field soil to the compost and...
vermiculite mixture into which nonmycorrhizal bahiagrass (*P. notatum* Flugge) seedlings were transplanted. The second tested the inoculation of the bahiagrass seedlings with AM fungi prior to transplant by a preliminary growth phase in potting media containing field-collected soil.

The first experiment to propagate indigenous AM fungi was conducted during the 2007 growing season at The Rodale Institute, Kutztown, PA. Seven gallon (26.5 L) black plastic bags (“Grow bags”, Worm’s Way, Bloomington, IN 47404) were filled with approximately 20 L of a 1:4 [v/v] mixture of compost and vermiculite, respectively. The compost was produced from yard clippings and leaves in windrows by the Lehigh Valley Compost Facility, Allentown, PA. The compost was first sieved to pass through a 1 cm mesh, and heat pasteurized on two consecutive days for approximately 8 h each. The latter was done to try to kill any AM fungi in soil introduced into the compost during turning.

Field soil was collected from the top 10 cm from a field under organic management at The Rodale Institute. A most probable number bioassay (MPN) (*Alexander*, 1965) of this soil indicated that there were 12 propagules cm⁻³. Four levels of soil addition were examined, each with three replicate bags. 0, 100, 200, or 400 cm³ of this soil was mixed into the individual bags of compost and vermiculite mixture just prior to transplanting three-month-old nonmycorrhizal bahiagrass seedlings, at a rate of five per bag, on June 20, 2007. The seedlings had been grown in a greenhouse in 65 cm³ conical plastic pots (RLC 4 ´ pine cell”, Stuewe and Sons, Corvallis, OR 97333) in an autoclaved mixture of sand, soil, vermiculite, and surface [SSVT] (Applied Industrial Materials, Corp., Deerfield, IL, 60015) (1:0.75:1:0.75 v/v). For these experiments and all others, bags were weeded and watered, as needed, throughout the growing season.

The second experiment to propagate indigenous AM fungi was conducted during the 2008 growing season. Bags of compost and vermiculite mixture were prepared as above. However, in this experiment the bahiagrass seedlings were grown for 3 months in a mixture of field soil and SSVT (1:5 v/v). The field soil was collected from the top 10 cm of the Farming Systems Trial at The Rodale Institute. Clearing and staining of the root systems of five of these seedlings with field soil addition in the experiments conducted in 2007, were pooled prior to analysis to yield just one MPN assay per treatment combination for these experiments. Space and labor constraints precluded conducting replicate MPN assays for that experiment (*Alexander*, 1965). Three MPN bioassays were conducted each for the comparison of inoculum production using seedlings grown in the greenhouse with field soil inoculum vs. two inoculated AM fungi (*G. mosseae* and *G. claroideum*). Most probable number bioassays utilized successive tenfold dilutions from 10⁻¹ to 10⁻³ using the sterilized SSVT mixture and bahiagrass seedlings as the host plant. Plants were grown in a controlled environment (day/night: 16/8 h, 25/18 °C, 60/70% R.H.) for four weeks.

Spores of AM fungi were quantified in 50 cm³ subsamples of media collected from each bag via wet sieving and centrifugation (*Jenkins*, 1964; *Gerdemann* and *Nicolson*, 1963). Roots were collected from the sample bags, rinsed, cleared and stained using trypan blue (*Phillips* and *Hayman*, 1970). Percentage root length colonized by AM fungi was quantified using the gridline intersect method using a dissecting microscope at 20× magnification (*Newman*, 1966). Experimental units were arranged according to a completely randomized design. Data were subjected to analysis of variance after square root (X + 1) (spore count and MPN) or arcsin (percentage root length colonized) transformation. Parameters for which significant treatment effects were found were characterized further using Tukey’s method of multiple comparisons to separate means (*α* = 0.05).

### 3. Results

#### 3.1. Propagation of indigenous isolates of AM fungi

Addition of field soil to the inoculum production system successfully propagated indigenous AM fungi (*Fig. 1*). AM fungi present in soil turned into the compost during its production evidently survived the heat treatment to colonize and increase, however survival was inconsistent with only 0.7% root length colonized in one bag and 19% in another, compared to an overall average above 60% root length colonized in the bags receiving field soil. Colonization of roots in the bags receiving soil was significantly greater than those not receiving soil, and not different among themselves (Pr > f < 0.0001). Propagule production ranged from 21.5 cm⁻³ (0.4 × 10⁶ per bag) in the uninoculated treatment to...
465 propagules cm\(^{-3}\) (8.8 \( \times \) 10\(^6\) per bag) for the treatment receiving 100 \( \times \) cm\(^3\) of soil. The latter represented an over 7000 fold increase of propagules relative to the number contained in the original soil.

Growth of bahiagrass seedlings in SSVT amended with field soil prior to transplant into the on-farm inoculum production system also successfully produced inoculum of AM fungi (Fig. 2). G. mosseae and G. claroideum together constituted a slight majority of the spores produced in these bags (40 \( \pm \) 7 and 27 \( \pm \) 12 spores 50 cm\(^{-3}\), respectively of an average of 132 \( \pm \) 26 total spores 50 cm\(^{-3}\)). Spore production by these species in bags containing plants preinoculated specifically with G. mosseae or G. claroideum was much greater than that in the bags growing the indigenous community (Table 1). However, quantification of the total amount of propagules of AM fungi in the compost and vermiculite mixtures indicated no statistically significant difference (Pr > f = 0.5990) among the three groups (Fig. 2). Colonization of roots after frost killed the bahiagrass was significantly greater in bags propagating the indigenous AM fungus community than those specifically inoculated with G. mosseae (71 \( \pm \) 3\% vs. 59 \( \pm \) 4\% root length colonized, respectively, Pr > f = 0.0456) and not different from those inoculated with G. claroideum (65 \( \pm \) 4\% root length colonized).

3.2. Alternate diluents for compost

Inocula of all AM fungi tested were successfully produced using perlite and horticultural potting media, in addition to the routine use of vermiculite, as diluents of compost in the on-farm system (Table 2). Populations of spores of the four fungi were not significantly different among the three diluents. Colonization of roots by G. mosseae was greater in perlite than in vermiculite, and colonization by G. claroideum was greater in perlite and vermiculite than in horticultural potting media. Colonization levels were not significantly different across diluents for the other fungi tested. Even though spore populations and colonization of roots were similar across diluents, vermiculite tended to produce more propagules than the other diluents as measured via MPN bioassay.

4. Discussion

Production of AM fungus inoculum on-the-farm potentially offers several important advantages over commercially available inocula. These have been detailed earlier (Douds et al., 2005, 2006) and include lower cost and potentially more taxonomic diversity than commercially purchased inocula. The current modifications allow for another benefit: the production of an inoculum containing locally adapted isolates of AM fungi.

Adoption of on-farm production of AM fungus inoculum by growers requires a greater degree of flexibility than that present in the original method. The original method required that compost be diluted with vermiculite and that the original starter inoculum be in the form of purchased bahiagrass seedlings colonized by spe-

### Table 1

<table>
<thead>
<tr>
<th>AM fungus Inoculation treatment</th>
<th>Pr &gt; f</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. mosseae</td>
<td></td>
</tr>
<tr>
<td>G. claroideum</td>
<td></td>
</tr>
<tr>
<td>Field soil</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>AM fungus</th>
<th>Perlite</th>
<th>Potting media</th>
<th>Vermiculite</th>
<th>ANOVA (Pr &gt; f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spores 50 cm(^{-3})</td>
<td>346 ( \pm ) 71</td>
<td>512 ( \pm ) 217</td>
<td>525 ( \pm ) 55</td>
<td>0.5993</td>
</tr>
<tr>
<td>Glomus mosseae</td>
<td>186 ( \pm ) 41</td>
<td>123 ( \pm ) 59</td>
<td>71 ( \pm ) 10</td>
<td>0.2130</td>
</tr>
<tr>
<td>Glomus sp.</td>
<td>290 ( \pm ) 77</td>
<td>650 ( \pm ) 133</td>
<td>324 ( \pm ) 97</td>
<td>0.0722</td>
</tr>
<tr>
<td>Glomus intraradices</td>
<td>452 ( \pm ) 135</td>
<td>1220 ( \pm ) 506</td>
<td>946 ( \pm ) 247</td>
<td>0.3048</td>
</tr>
<tr>
<td>Glomus claroideum</td>
<td>69.1 ( \pm ) 6.4</td>
<td>56.4 ( \pm ) 2.9</td>
<td>46.6 ( \pm ) 0.8</td>
<td>0.0120</td>
</tr>
<tr>
<td>Glomus mosseae</td>
<td>59.9 ( \pm ) 4.7</td>
<td>60.9 ( \pm ) 4.9</td>
<td>64.6 ( \pm ) 5.2</td>
<td>0.7804</td>
</tr>
<tr>
<td>Glomus sp.</td>
<td>67.9 ( \pm ) 6.1</td>
<td>65.8 ( \pm ) 2.5</td>
<td>81.1 ( \pm ) 2.5</td>
<td>0.0534</td>
</tr>
<tr>
<td>Glomus intraradices</td>
<td>72.4 ( \pm ) 32</td>
<td>58.3 ( \pm ) 2.4</td>
<td>70.9 ( \pm ) 2.2</td>
<td>0.0080</td>
</tr>
<tr>
<td>Glomus claroideum</td>
<td>36.5</td>
<td>21.5</td>
<td>120.0</td>
<td></td>
</tr>
<tr>
<td>Glomus mosseae</td>
<td>21.5</td>
<td>12.0</td>
<td>46.5</td>
<td></td>
</tr>
<tr>
<td>Glomus sp.</td>
<td>21.5</td>
<td>46.5</td>
<td>145.0</td>
<td></td>
</tr>
<tr>
<td>Glomus intraradices</td>
<td>46.5</td>
<td>21.5</td>
<td>46.5</td>
<td></td>
</tr>
</tbody>
</table>

\* Means of four observations for spore populations and colonization, results of one pooled sample for propagules (most probable number bioassay).
cific isolates of AM fungi. These characteristics are restrictive, especially the latter. Experiments conducted here demonstrated that these restrictions are readily overcome.

Inoculum of AM fungi was successfully produced in compost mixed with vermiculite, perlite, or horticultural potting media (Table 1). However, propagule numbers tended to be greater in the vermiculite based media. Propagules of AM fungi consist of spores, colonized root pieces with vesicles, and infective hyphal fragments. The laminar sheets of vermiculite may provide an environment conducive to the growth and persistence of AM fungus hyphae. The similar spore populations and colonization of roots among the three media amendments support this idea.

The production of inoculum using perlite as the diluent offers an added benefit. Among the farmers targeted by the on-farm inoculum production method are vegetable farmers who grow their own seedlings for later transplanting to the field. The goal is to mix the inoculum into the horticultural potting media in which the seedlings are grown. An inoculum consisting primarily of perlite stands out in greater contrast to the predominantly dark colored potting media than does a vermiculite and compost based inoculum. This aids in mixing the inoculum homogeneously throughout the potting media.

Two methods were used successfully to produce inocula of indigenous isolates of AM fungi. Inocula were produced both by (1) mixing field soil into the compost and vermiculite mixture in the production bags (Figs. 1 and 2) pre-colonizing the bahaagrass seedlings during the preliminary greenhouse growth phase by adding a small amount of field soil to the SSVT mix (Table 1, Fig. 2). In addition to making the method more practical, there are several reasons why the production and utilization of the indigenous community of AM fungi can be desirable. First, there is some evidence that the indigenous community of AM fungi can be more effective in promoting the growth of plants in its native soil than introduced isolates (Sreenivasas, 1992; Oliveira et al., 2005). Secondly, functional diversity has been demonstrated among AM fungi in characteristics such as glomalin production (Wright and Upadhyaya, 1996), variability in growth response among plant species (Pope et al., 1983), spatial exploration of the soil for P (Smith et al., 2004), and patterns of colonization (Hart and Reader, 2002). This makes it beneficial to have a taxonomically diverse inoculum, which was accomplished when the bahaagrass was inoculated with field soil.

Some degree of the diversity of the original community will be lost, however, for a number of reasons. AM fungi are sometimes patchy in distribution, and may not be present in the soil used to inoculate the bahaagrass (St. John and Koske, 1988). In addition, even though bahaagrass is a very good general host for AM fungi (Struble and Skipper, 1985), use of the bahaagrass monoculture will tend to select for those AM fungi among the community which propagate best with that particular host (Hetrick and Bloom, 1986; Giovannetti et al., 1988). This is the primary reason why, in the original method (Douds et al., 2006), different species of AM fungi are propagated in separate bags rather than all together in the same bag. This avoids competition among the isolates for occupancy of the roots and, concomitantly, for the fixed carbon needed for growth. The species diversity in the original method comes from mixing inocula of various species together at the time of harvest and utilization of the inoculum. Since AM fungus species diversity and plant community diversity are correlated (van der Heijden et al., 1998), the potential weakness in the propagation of indigenous AM fungi via the on-farm methods developed here could be combated by using several different host plants. This option has not been explored.

The combination of these modifications to the on-farm system, e.g. the propagation of indigenous isolates of AM fungi in a perlite plus compost mixture, was not tested explicitly. Since the starting material in both systems, i.e. colonized bahaagrass seedlings in SSVT mix, was the same and propagated AM fungi well in perlite, vermiculite, or potting media plus compost mixtures, it should not matter whether the AM fungi colonizing the bahaagrass was a selected isolate or members of the indigenous community.

The naturally-occurring symbiosis between AM fungi and the roots of most crop plants is a potentially powerful tool in sustainable agricultural systems. Agronomists and horticulturists now have greater flexibility for the on-farm production of AM fungus inoculum in temperate climates.

Acknowledgements

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References


Oliveira, R.S., Vosátka, M., Dodd, J.C., Castro, P.M.L., 2005. Patterns of colonization (Hart and Reader, 1996) and functional diversity have been demonstrated among AM fungi in characteristics such as glomalin production (Wright and Upadhyaya, 1996), variability in growth response among plant species (Pope et al., 1983), spatial exploration of the soil for P (Smith et al., 2004), and patterns of colonization (Hart and Reader, 2002). This makes it beneficial to have a taxonomically diverse inoculum, which was accomplished when the bahiagrass was inoculated with field soil.

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