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SHORT NOTE

Evaluation of commercial arbuscular mycorrhizal inocula in a sand/peat medium

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Abstract Eight commercial inocula of arbuscular mycorrhizal fungi (AMF) were tested for their ability to colonize plant roots in the sand/peat medium specified by the U.S. Golf Association for use in putting greens. Using the standard assay for potency of inocula (*Zea mays* grown for 6 weeks in containers), inocula were added at the rate recommended by the manufacturer as well as at five and ten times the recommended rate. To ensure that growth conditions were conducive to AM formation, a soil-based inoculum of native AMF also was assessed for inoculum potential. Only three of the commercial inocula formed mycorrhizas when used at the recommended rate, and the extent of colonization ranged from 0.4 to 8%. Increasing the amount of inoculum resulted in colonization levels of 8.6 to 72.5% at the highest rate (10×). Mean colonization using the native AMF was 60%. One inoculum that did not form mycorrhizas at the recommended rate or at 5× produced 8.6% colonization at 10×. An inoculum that did not produce mycorrhizas at any application rate did contain a fungus tentatively identified as a root pathogen (*Olpidium brassicae*) that colonized the corn roots. The failure of five of the eight commercial inocula to colonize roots when applied at the recommended rate suggests that preliminary trials should be made before commercial AMF inocula are used in important plantings.

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Introduction

Arbuscular mycorrhizal fungi (AMF) form mutualistic associations with the majority of plant species (Smith and Read 1997). Hyphae of AMF grow into the soil from roots and greatly improve access to immobile nutrients (especially P, but also Cu and Zn; Miyasaka and Habte 2001) as well as inorganic N (Govindarajulu et al. 2005), reducing fertilizer requirements (Gemma et al. 1997a). AMF have been shown to increase plant growth, increase chlorophyll and leaf P content, and greatly improved tolerance to drought and salinity stress (Sylvia et al. 1993a; Gemma et al. 1997b; Auge 2001; Koske and Gemma 2005). In addition, AMF may provide protection against some root pathogens (e.g., Caron 1989; Azcon-Aguilar et al. 2002; Whipps 2004).

While many of the benefits of AMF to plants are known, specific steps must sometimes be taken to ensure that roots are colonized by these fungi soon after seeding or transplanting to get maximum results from the association. When seedlings or transplants are grown in soil lacking AMF or with a low population of AMF, addition of inocula can induce marked increases in survival, establishment, and growth (Bethlenfalvay 1992; Clapperton and Reid 1992; Gemma et al. 1997a; Koske and Gemma 1997). Because the large underground spores of AMF are relatively poor dispersers in comparison to many other fungi, soils with low populations of AMF are likely to remain so for long periods of time (Koske and Gemma 1997).

The significance of these studies has not been ignored by the commercial sector, and there are now numerous

manufacturers offering AMF inoculum for sale (Gianinazzi and Vosátka 2004). Although most manufacturers indicate the potency of their product by listing the concentration of spores or infective propagules of the fungi in their product, the actual root-colonizing ability of these inocula is not known. In a recent evaluation of the performance of ten commercial inocula in nursery potting mixes, half failed to form mycorrhizas with the test plants (Corkidi et al. 2004). Comparisons between field-collected and commercial inocula performed by Gaur et al. (1998) and Rowe et al. (2007) found that commercial inoculum underperformed in almost all cases. The goal of the present study was to perform a preliminary screening of the mycorrhizal inoculum potential (MIP) of eight commercial AMF inocula using *Zea mays* L., the standard assay species (Moorman and Reeves 1979; Corkidi et al. 2004). From our earlier experiences with the variability in commercial inocula and their occasional failure to colonize at the recommended rates of application, three rates were assessed (see below).

Materials and methods

Eight commercial inocula were purchased in granular form and stored under conditions specified on their labels. To ensure the anonymity of the products, each inoculum was assigned a number (1–8), and these numbers are used in this study. Suppliers were not told that their inocula were to be evaluated and compared to other inocula. All experimentation was performed within 1 month of receiving the inocula and before the expiration date for all products. Application rates were as recommended by the producers (Table 1). Six of the eight manufacturers provided a recommended rate for use with Turfgrass. For the other two inocula, we used the rate recommended for general use. The infectivity (MIP; Moorman and Reeves 1979) of the commercial AMF inocula and of three samples of sand dune soil that we routinely use as inocula in growth studies was assessed in a growth-room study. MIP assays were performed in tapered plastic containers (Super Cells®; Steuwe and Sons, Corvallis, OR 97333) measuring 20.7 cm tall×3.8 cm diameter and containing 165 ml of the growing medium. Our interest in mycorrhizae in golf putting greens led us to perform the studies in a growing medium recommended for use in greens by the U.S. Golf Association (Bengeyfield 1989). The medium was composed of a 4:1 (*v*/*v*) of quartz sand and milled Canadian Sphagnum peat (Gemma et al. 1997a). The pH of the growing medium was adjusted to 6.2 by adding pulverized lime, and 0.046 g of a granular starter fertilizer (13-25-12, Agway, Syracuse, NY 13221) was incorporated into the top 12 mm of each container immediately before planting. A small piece of plastic window screen was inserted into the bottom of each container before filling to prevent the contents from falling out the drainage holes. All treatments and controls were prepared in this manner.

Three inoculation rates were tested for each commercial inoculum: the recommended rate (Table 1) and 5× and 10× the recommended rate. Previous studies with commercial inocula (Koske and Gemma, unpublished observations) had indicated that the rate recommended by the manufacturers sometimes is too low for mycorrhizas to form within a reasonable time, and we included the 5× and 10× rates to determine if the absence of AM formation at the recommended rate was caused by an insufficient number of propagules of AMF in the inoculum. Inocula for each treatment were thoroughly mixed in 1 l of growing medium, and five containers were filled. Controls were prepared for each inoculum. For these, inoculum (at the recommended rate) was microwaved for 2 min to eliminate any viable propagules (Ferriss 1984) and added in place of untreated inocula when the containers were being prepared. Each of the commercial inocula was tested at three inoculation rates and replicated five times. The controls were replicated three times.

To insure the effectiveness of the bioassay (growing medium, watering schedule, fertilizer amount, time of harvest, and staining protocol), the MIP of field-collected inoculum was run concurrently. This inoculum consisted of sand collected from the root zones of three plants of American beachgrass (*Ammophila breviligulata* Fern.) growing at the crest of a sand dune in South Kingstown, RI. The collected soils were examined to confirm the presence of spores of AMF. Previous studies had shown that addition of 10 ml per container of sand dune inoculum produces a high level of AMF colonization in plant roots (Koske and Gemma, unpublished observations), and was the only rate of application tested in this experiment.

Table 1 Calculated number of propagules per container used in this study based on data from product labels of commercial AMF inocula and added at the recommend rate

Product[a]	1	2	*3*	4	5	*6*	*7*	8
No. of propagules	96	0.13	7	6	ns	ns	ns	27–67

ns Not stated

[a] Products italicized produced colonization at recommended rate.

Seeds of *Z. mays* var. "Golden Cross" were germinated for 3 days between moist paper towels at 25°C, and a single seedling was planted in each container. Plants were grown for 6 weeks (a duration sufficient to allow development of colonization; Corkidi et al. 2004) in an air-conditioned growth room at 24–33°C and illuminated with a 1,000-W metal halide bulb (approximately 350–500 μein) for 14 h day^{1}. Plants were watered as needed (approximately every other day) with deionized water. No additional fertilizer was added during the first 4 weeks of growth. Four weeks after transplanting, the watering regime was changed. Instead of receiving deionized water as needed, plants were watered with a dilute fertilizer solution prepared from a complete fertilizer that included micronutrients (HI-CAL peat-lite 20-0-20; Grace-Sierra Horticultural Products, Milpitas, CA 95035) amended with $MgSO_4$. This watering solution contained N (25 ppm), K (21 ppm), Ca (7.5 ppm), SO_4 (2.34 ppm), Mg (0.59 ppm), B (0.050 ppm), Cu (0.025 ppm), Fe (0.025 ppm), Mn (0.014 ppm), Mo (0.003 ppm), and Zn (0.004 ppm). The pH of this solution was adjusted to 6.3 with KOH.

At harvest, the root system of each plant was washed, cleared, and stained for analysis of root colonization by AMF (Koske and Gemma 1989), and extent of coloni zation was measured using the grid-line intersect method (Giovannetti and Mosse 1980). Roots of control plants were stained and examined to insure the absence of AMF colonization. Frequency of AMF in inoculated plants was calculated by dividing the number of plants in each treatment that had formed mycorrhizas by the total number of plants in the treatment (five for commercial inocula, three for dune inocula). The result is expressed as a percentage.

Table 1 lists the abundance of spores in the inoculum (as supplied by the manufacturer) and the recommended rate of application. From this information, we calculated the number of spores that would be in the assay container when added at the recommended rates (Table 1). Three of the inocula (5, 6, and 7) did not come with information on the concentration of spores.

Root colonization percentages underwent arcsin transformation before statistical analysis. Data were then analyzed using analysis of variance and linear regression (Statview, SAS), and significance was assigned at $P<0.05$.

Results

Only half of the commercial inocula were capable of forming mycorrhizas under the conditions of this study. At the recommended rate, only three inocula (3, 6, and 7) colonized roots, and percentages of root colonization were very low (0.4 to 8.0%; Table 2). Frequency of colonization with these inocula ranged from 10–80%. The extent of root colonization and frequency of AMF in plants increased significantly at the 5× rate for inoculum 3 ($P<0.0001$), with a colonization level of 68.6%, but did not increase significantly in plants inoculated with inoculum 7 ($P=0.1803$), with a colonization level of 4.9%. Plants inoculated with inoculum 6 showed a significant decrease ($P=0.0189$) in colonization levels at the 5× rate. At 10×, the recommended rate inocula 3, 7, and 8 produced significantly more colonization than at the recommended rate ($P<0.0001$, $P=0.0169$, and $P<0.001$, respectively), and 80–100% of plants had formed mycorrhizas. Inoculum 8 produced mycorrhizas only at the 10× rate, with 100% of plants colonized and a colonization level of 8.6%. No colonization occurred in plants inoculated with inoculum 6 at the 10× rate, although colonization did occur at the recommended rate and at 5×. Four of the eight commercial inocula (1, 2, 4, and 5) did not form AM at any application rate (Table 2). The plants grown with each of three sand dune inocula had colonization levels of 46 to 74%, and all plants were colonized (Table 2).

Inocula 3 and 7 showed significant linear relationships between inoculation rate and extent of colonization ($r^2=0.95$, $P<0.0001$ and $r^2=0.608$, $P=0.0398$, respectively). Inoculum 4 (which did not form mycorrhizas at any application rate) did produce a root infection by a fungus tentatively identified as *Olpidium brassicae*, a common root pathogen. The extent of colonization of roots by this fungus increased with increasing amounts of inoculum. Because *O. brassicae* forms large intraradical sporangia that resemble vesicles, the stained roots containing *O. brassicae* could easily be mistaken for AMF by an untrained observer.

Discussion

Overall, the commercial inocula tested were incapable of much colonization when used at the recommended application rate under the test conditions, and half of the inocula were unable to form AMF even at the highest rate. Even at highest application rates, levels of colonization comparable to those using the sand dune inoculum were achieved only by inoculum 3. Our findings on the variation in inoculum quality are in general agreement with those of Corkidi et al. (2004) who tested ten commercial inocula (provided by producers who agreed to have their inocula tested) at the recommended rate using *Z. mays* as the host. Three growing media (differing greatly in their physical and chemical properties) were used in that study, a soil/sand (1:1) mix being most similar to the one used in the present study. After 6 weeks, six of the ten inocula had formed mycorrhizas with corn roots, with colonization levels ranging from 1 to 32% in the sand/soil mix.

Table 2 Effects of different application rates of commercial AMF inocula on percentage of root length colonized and frequency of AMF formation in plants of *Zea mays*

Inoculum #	Recommended rate		5× Recommended rate		10× Recommended rate	
	% Col.	Freq. (%)	% Col.	Freq. (%)	% Col.	Freq. (%)
1	None	None	None	None	None	None
2	None	None	None	None	None	None
3	6.1±3.0[a]	80	68.6±4.4	100	72.5±3.0	100
4	None	None	None	None	None	None
5	None	None	None	None	None	None
6	8.0±4.4	80	1.2±1.2	20	None	None
7	0.4±0.4	10	4.9±2.7	60	12.5±6.3	80
8	None	None	None	None	8.6±2.9	100
Dune 1	60.5±2.9	100	nt[b]	nt	nt	nt
Dune 2	73.7±1.2	100	nt	nt	nt	nt
Dune 3	46.4±3.0	100	nt	nt	nt	nt

[a] Mean±SD
[b] Not tested

Major determinants of the ability of inocula to colonize include the abundance of spores and other infective propagules (e.g., colonized root fragments, pieces of hyphae) in the inoculum, the viability of the propagules, the amount of inoculum that is used, the ability of the plant species to form mycorrhizas, and abiotic factors (e.g., light, temperature, soil nutrients, and pH; Smith and Read 1997). A low propagule count in the inoculum will often result in an increased lag period before colonization occurs and a lower extent of colonization during the establishment phase of growth (Haas and Krikun 1985; Khan 1988; Clapperton and Reid 1992; Sylvia et al. 1993b). The recommended rates for several of the inocula tested in this study (i.e., 2, 3, and 4) supplied only 0.13 to 7 propagules to the growing medium in each container (based on propagule density provided by the manufacturer; Table 1). Assuming that the stated propagule densities are accurate, such low levels would be unlikely to result in extensive colonization in 6 weeks. The increase in extent and frequency of colonization that accompanied using 5× and 10× the recommended rate (inocula 3, 7, and 8) suggests that propagule numbers were too low for the 1× rate to be effective over the study period. The decline in colonization by inoculum 6 as the application rate increased may have resulted from inhibition by other components in the inoculum, possibly affecting pH when used at levels in excess of the recommended rate. The high level of colonization by the sand dune inocula probably resulted from the large amount of inoculum added per cone, equivalent to approximately 60 ml l^{1} (approximately 90 g l^{1}), far greater that the commercial inocula.

For those inocula that claimed to have high propagule densities but had low or no ability to colonize, the likely causes were: far fewer spores or propagules were present than claimed, dead or dormant propagules, and/or fungi unsuited to the growth medium (see below). In previous studies, several commercial "inocula" that were examined contained no spores at all, despite claims of the manufacturer (Koske, unpublished observations).

Edaphic factors have been shown to greatly affect AMF function and association with host plants (Bentivenga and Hetrick 1992; Sylvia and Williams 1992), and some species of AMF tend to be more effective at colonizing in certain soils or growing media than others (Lambert et al. 1980; Gianinazzi-Pearson et al. 1985; Henkel et al. 1989; Stahl and Christensen 1991; Sylvia et al. 1993a, b). In the assessment of inocula by Corkidi et al. (2004), levels of colonization differed depending upon the identity of the inoculum as well as the composition of the growth medium. However, the composition of the medium never completely prevented mycorrhizal formation, and inocula that were unable to colonize in one growth medium were unable to colonize in the other two media. The generally poor performance of inocula when used at the recommended rate in the current trial may reflect the inability of the AMF to vigorously colonize roots in the sand/peat medium unless a sizable population of AMF propagules is present. Inclusion of peat into growing mixes has been shown to reduce the extent of root colonization as well as to decrease growth responses to AMF by host plants even when used at concentrations of peat as low as in the present study, but peat has not been shown to completely prevent colonization (Biermann and Linderman 1983; Linderman and Davis 2003; Koske and Gemma 1995). Based on the results of Corkidi et al. (2004), the failure of four of the inocula to colonize at any application rate in the present study is more likely to reflect problems occurring during production of the inocula rather than the composition of the growth medium used in this test.

Our findings and those of Lovato et al. (1992), Gaur et al. (1998), Duffy and Casselles (2000), Corkidi et al. (2004), and Rowe et al. (2007) suggest that commercial inocula will vary greatly in their ability to perform in different growing mixes and with different host plants. For

those inocula that were capable of colonization (inocula 3, 6, 7, and 8) application rates may need to be five or ten times higher than that recommended by the manufacturer when used in a sand/peat medium such as USGA putting greens or peat-containing greenhouse media. Claims by manufacturers notwithstanding, it may be necessary to run a small trial (including a non-inoculated control) before using commercial inocula for large plantings or important applications.

This study also raises concerns about the quality control practices implemented by many inoculum producers. With the precariousness of inoculum viability, the potential loss of desired AMF species in inoculum through subsequent culturing, and the possibility for contamination of stock cultures by pathogens, a standardized method of screening or certification of effectiveness should be considered before distribution to customers. Such a system could lead to greater product confidence and the prevention of the distribution of low quality inoculum.

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