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CAN COMMERCIAL AMF INOCULUM IMPROVE PRAIRIE RESTORATION? COLONIZATION AND GROWTH EFFECTS OF FUNGI ON SAND PRAIRIE PLANTS AND SMOOTH BROME IN FIELD SOILS.

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CAN COMMERCIAL AMF INOCULUM IMPROVE PRAIRIE RESTORATION? COLONIZATION AND GROWTH EFFECTS OF FUNGI ON SAND PRAIRIE PLANTS AND SMOOTH BROME IN FIELD SOILS.

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ABSTRACT

Paluch, E.C. Can commercial AMF inoculum improve prairie restoration? Colonization and growth effects of fungi on sand prairie plants and smooth brome in field soils. MS in Biology, May 2011, 61 pp. (T. Volk and M. Thomsen)

Arbuscular mycorrhizal fungi (AMF) affect the species composition, structure, and function of prairie ecosystems, but prairie restorations are frequently located in sites with depauperate AMF communities. In this greenhouse study, four native sand prairie species (*Schizachyrium scoparium, Elymus canadensis*, *Monarda punctata*, and *Aster ericoides*) and an invasive grass *(Bromus inermis)* were grown in unsterilized field soils and treated with two brands of commercial AMF inoculum. Inocula were applied at the manufacturers' suggested rate and at two times the recommended rate. Soil was collected from two locations: a meadow enrolled in the Conservation Reserve Program (CRP) that is dominated by *Bromus inermis*, and from an active agricultural field. At the rates used in this study, the commercial inoculum did not significantly increase biomass or percent colonization of any grass species. However, both biomass and percent colonization in *Aster* were influenced by inoculum type, although treated individuals were not significantly different from control groups in pairwise comparisons. Increased percent colonization in all three grasses and increased biomass in the native grasses was observed in individuals grown in the CRP soil. In terms of prairie restoration, an existing soil fungal community appears to be more productive in terms of percent colonization and plant biomass than an addition of commercial inocula.

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CHAPTER I

PRAIRIES, RESTORATION, AND MYCORRHIZAE

Prairie Restoration

Prior to European settlement of North America, prairies covered roughly 975 million acres in the heart of the continent (Lemon 1968). The prairies were large, grass-dominated expanses that received too little rainfall to support trees (Transeau 1935; Collins 1990). Frequent fires also prevented the establishment of most trees, while promoting the growth of grasses (Transeau 1935; Sauer 1950). Today, the prairie region is typically categorized into three smaller subgroups that run from east to west: tallgrass prairie, mixed grass prairie and short grass prairie. Rainfall decreases along the east-west gradient, with the tallgrass prairie receiving the most rain, and short grass receiving the least (Lemon 1968). The tallgrass prairies are known for the deep, nutrient-rich black soils that formed over hundreds of years from the decomposition of prairie vegetation (Smith 1990; Kline 1997). The fertile soils of the tallgrass prairies are favored for agriculture, and with the invention of the steel plow, prairie was quickly converted into farm fields (Samson et al. 2004). It is currently estimated that only one hundredth of one percent of the original tallgrass prairie remains, most having been converted to corn and soybean production (Smith 1990; Neely and Heister 1987). As a result, North American prairies are one the most endangered ecosystems in North America and in the world (Samson and Knopf 1994).

Sand prairies are a subset of the tallgrass prairie. Prior to European settlement, sand prairies existed along major river ways in southwestern Wisconsin, including the Mississippi, Wisconsin, and Black Rivers (Wisconsin Department of Natural Resources 2005). Soil in sand prairies is typically acidic and the deep, dark A horizon that typifies tallgrass prairies may be absent in the driest locations (White and Madany 1981; Bowles et al. 2003). Dry conditions and the poor soil quality of sand prairies leads to the overall reduction of vegetation height. *Schizachyrium scoparium* (Little Bluestem), *Stipa spartea* (Porcupine Grass), and *Koeleria macrantha* (June Grass) are the dominant grasses of the sand prairie. Characteristic forbs include *Monarda punctata* (Spotted Beebalm), *Euphorbia corollata* (Flowering Spruge), *Liatris aspera* (Rough Blazingstar), and *Helianthus occidentalis* (Western Sunflower) (White and Madany 1981; Betz and Lamp 1992; Bowles et al. 2003). Wisconsin sand prairies are associated with several species of concern including eleven reptiles, ten species of birds, and three mammals (Wisconsin Department of Natural Resources 2005). Like the other tallgrass prairies, sand prairies are under pressure from exotic species such as *Euphorbia esula* (Leafy Spurge) and *Centaurea biebersteinii* (Spotted Knapweed), woody plant invasion, fire suppression and conversion to agriculture.

In response to the rapid loss of the prairie ecosystem, many people have worked to preserve remaining prairie parcels. Ecologists started experimenting with the process of returning agricultural fields and degraded prairies into functional prairie ecosystems as early as the 1930’s. From the small beginning at Curtis Prairie at the University of Wisconsin Arboretum in 1934, a whole movement and science of prairie restoration was born (Wegener et al. 2008; Anderson 2009). As the science of prairie restoration has matured, prairie restorations have been started at every level, from research facilities, federal, state and municipal properties, to schoolyards and private landowners. Prairie restorations may range from small display gardens to hundreds of acres (Packard and Mutel 1997). Regardless of size, accurately re-creating the complex prairie ecosystem requires careful planning and implementation of a combination of restoration techniques.

Over the years a basic methodology has developed for restoring prairies. Schramm (1990) and Kline (1997) have provided detailed overviews of typical restoration procedures. After a site has been chosen, most restorations begin with reviewing public land survey notes, reference sites, herbarium records, letters, pioneer journals, or other sources for pre-settlement vegetation information. Soil type data is also used to better determine what species are appropriate at a certain site. With these various sources of information, a plant species mix is designed. Prior to planting, the existing plant community and soil condition of the restoration site need to be evaluated. Sites that have been tilled for agricultural purposes are generally regarded as relatively blank slates and prairie seeds may be easily planted. If the site is pasture or weedy it may be herbicided and/or plowed prior to planting to remove woody or invasive species. In contrast, if the property has some desirable species, interseeding prairie seeds into the existing vegetation is an option. The seeds needed for the restoration may be gathered, purchased, or a combination of the two, and planted either by hand or with a specialized seed drill. In the first three years after planting, restorations are managed by prescription burns, herbicide, and/or mowing to suppress weeds and thereby encourage the growth of prairie plants.

As a result of research into pre-settlement vegetation and years of experimentation, restored prairies are beginning to resemble prairie remnants in terms of their plant species composition. The ultimate goal of any restoration, however, is to produce a prairie ecosystem that also resembles a natural prairie in terms of structure and function. Two factors that greatly influence structure and function are disturbance and competition. Fire and grazing are the most important forms of disturbance in historical prairie sites (Martin et al. 2005). Prairie plants have evolved to survive if not thrive with periodic burns, which increase light availability by reducing duff and introduce a large flush of nutrients (Collins 1990). Fires occurred frequently across the pre-settlement prairie (Hulbert 1986). During the summer, dry grasses would ignite easily and burn hundreds of acres at a time. In addition to naturally-occurring fire, Native Americans would set fires to eliminate woody plants and rejuvenate grassland areas to attract herds of bison and other large grazers (Transeau 1935; McHugh 1972; Collins 1990). Today, restorationists use prescription burns to mimic natural fire patterns. These burns are effective at controlling woody vegetation, reducing duff, and releasing nutrients (Howe 1994). However, the majority of prescription burns are conducted during the dormant season, while historically most fires occurred during the summer growing season (Howe 1994). The timing of a fire can favor or disadvantage different plant species, and therefore can change species composition, structure, and overall grassland structure. Dormant-season prescription burns benefit the dominant C_4 grasses, and often result in a taller, denser prairie structure and lower species diversity (Hulbert 1986; Howe 2000).

Hand in hand with fire, large ruminant herbivores influenced the pre-settlement prairie in ways that researchers are still working to understand (Knapp et al. 1999). Prior to 1800, an estimated 28 to 34 million bison grazed across the prairie (McHugh 1972; Flores 1991). Bison had a profound effect on the prairie (Truett et al. 2001). By preferentially grazing on the dominant C_4 grasses, bison provided a competitive advantage for the less dominant C_3 grasses and forbs (Knapp et al. 1999). As a result, areas where bison grazed frequently had a higher diversity of plant species (Collins et al. 1998). Bison also played a significant role in nutrient dynamics, through grazing and the deposition of plant-available nitrogen in their feces, urine, and carcasses (Steinauer and Collins 1995; Knapp et al. 1999; Truett et al. 2001). Although bison were likely a keystone species in prairie ecosystems, they are rarely found in restorations (Steuter 1997; Knapp et al. 1999). Most prairie restorations are too small or not mature enough to provide adequate habitat for a herd of bison, and management costs are prohibitive for most landowners (Martin et al. 2005; Steuter 1997).

Together with disturbance, prairie ecosystems are shaped by plant competition for light and nutrients. The presence of standing dead plant material and litter greatly affects both species composition and productivity by reducing light availability to live plants at the ground level and within the canopy (Knapp and Seastedt 1986). Standing dead remains of the dominant C_4 grasses can reduce light significantly for the surrounding forbs and C_3 grasses because they are typically taller (Turner and Knapp 1996). Therefore, in sites where grazing and fire are suppressed or absent, the C_3 grasses and forbs are generally outcompeted, resulting in tall, dense stands of the dominant C_4 grasses (Leach and Givnish 1996). In prairie restorations, standing litter can be reduced, and in turn, light penetration increased, through the introduction of disturbance in the form of burning, grazing and/or mowing. These management practices are critical to maintain proper structure and plant species composition in restorations.

As in most terrestrial ecosystems, nitrogen is the most common limiting nutrient in prairies (Wedin and Tilman 1996). Competition for nitrogen has been shown to influence tallgrass prairie species composition in both old-field sites and remnant prairies (Inouye et al. 1987; Baer et al. 2003). In general, higher nitrogen levels favor C_4 grasses both because of their nutrient uptake abilities and because light competition is generally more important in more productive areas (Turner and Knapp 1996, Baer et al. 2003). Nitrogen fixing plants are less common in high nitrogen soils, which leads to lower species diversity. As prairie restorations are often planted into former agricultural lands, the soil nitrogen content may have been altered drastically by fertilizer application or planting nitrogen fixing crops (Camill et al. 2004). The overabundance or depletion of nitrogen from the soil will influence the species composition of the restoration, and in turn prairie structure.

It is an understatement to say that the functional dynamics of the prairie ecosystem are complex. Species composition and structure of prairies are maintained by forces that work together like pieces in a giant jigsaw puzzle, simultaneously influencing the effects of multiple other factors. Just like a puzzle, a restoration that is missing pieces will not accurately resemble prairie in structure, function, or species composition. An additional piece of this restoration "puzzle" is the role played by mycorrhizal fungi.

Mycorrhizal Fungi

Mycorrhizal fungi are a diverse group of fungi that form mutualistic symbiotic relationships with plants. Approximately 90 to 95% of land plants form associations with some type of mycorrhizal fungi (Bago et al. 2000). The term mycorrhiza comes from the two Greek roots, “myco” meaning fungus and “rhiza” which translates to root. A mycorrhizal symbiosis involves one or more plants (the photobiont) and a fungus (the mycobiont). The fungi produce a vast network of hyphae that extend far beyond the rhizosphere of the photobiont and assist with water and nutrient uptake, especially phosphorus and nitrogen. In turn, the mycobiont is supplied with photosynthate in the form of sugar (Smith and Read 1997). The common classification of mycorrhizae comprises seven diverse groups of fungi, with the most common being ectomycorrhizal fungi (ECM) and arbuscular mycorrhizal fungi (AMF) (Brundett 2002).

Ectomycorrhizal fungi mainly form relationships with certain families of woody vegetation (such as species within Caesalpinioideae, Fagaceae, and Pinaceae) and are classified within the Basidiomycota, Ascomycota, and Zygomycota (Smith and Read 1997, Brundett 2002). There are currently over 6000 known species (Brundett 2002). Ectomycorrhizal fungi characteristically produce a hyphal sheath around the outside of the root, called a mantle, which can vary in thickness and color depending on the species and conditions (Smith and Read 1997). Identification of ectomycorrhizal fungi is often based on the morphological characteristics of the mantle. Hyphae extend beyond the mantle into the soil, which increases intake of water and nutrients. In addition, ECM form what is known as the Hartig Net, which facilitates water and nutrient exchange between the plant and fungus. Although the Hartig Net grows between the epidermal and between the cortical cells of the host root, the hyphae do not penetrate the root cells (Smith and Read 1997).

Conversely, arbuscular mycorrhizal fungi (AMF) extend into the cortical root cells, although, the hyphae do not break through the cell membrane (Bago et al. 2000). Arbuscular mycorrhizal fungi, also known as endomycorrhizal fungi and vesicular-arbuscular mycorrhizal (VAM) fungi, are the most common form of mycorrhizal symbiosis; with an estimated 200,000 host plants (Smith and Read 1997; Helgason et al. 2007). AMF form relationships with a wide variety of plants including angiosperms, gymnosperms, pteridophytes, and bryophytes (Harrison 1999; Bago et al. 2000). Although AM fungi are obligate symbionts, their plant hosts may range from mildly mycophilic (perform better when AMF are present) to completely myco-dependant (unable to survive without a mycobiont at some time in the host's lifecycle). A given species of plant may be colonized with multiple species of fungi at a given time. Likewise an individual fungus may be linked to several individual plants of the same or different species (Smith and Read 1997).

Despite the numerous plant species that form AM relationships, a relatively small number of arbuscular mycorrhizal fungi are known. Historically AMF classification was based on the development of the spore and the spore wall in addition to the morphological features of the spore (color, size, shape, etc.) (Smith and Read 1997). With the advancement of molecular techniques, AM fungi can be more accurately identified (Redecker and Raab 2006). Based on molecular phylogenies and morphological features, the approximately 200 named species of AMF are categorized within the ten genera in the phylum Glomeromycota (Redecker and Raab 2006). All AM fungi form aseptate hyphae, and approximately 50 to 80% of species produce sac-like vesicles in the root cortical cells that function in starch storage (Smith and Read 1997; Redecker and Raab 2006). AMF form large, (40-800 µm) multinucleate, asexual spores that may be borne singularly or in clusters. Sexual reproduction in AMF is unknown (Redecker and Raab 2006).

The name arbuscular mycorrhizal fungi comes from the signature formation of tree-like arbuscules that are believed to function in the bi-directional exchange of nutrients. Arbuscules are sections of hyphae that are dichotomously and copiously branched. The increased surface area created by the branching is believed to assist with nutrient exchange (Bago et al. 2000). In 1973, Ho and Trappe were the first to demonstrate the flow of carbon from plants to AM fungi through the detection of ^{14}C labeled photosynthate in the fungal hyphae and spores (Smith and Read 1997). It has been estimated that 4 to 20% of a plant's photosynthate may be funneled to fungal symbionts (Bago et al. 2000, Figure 1).

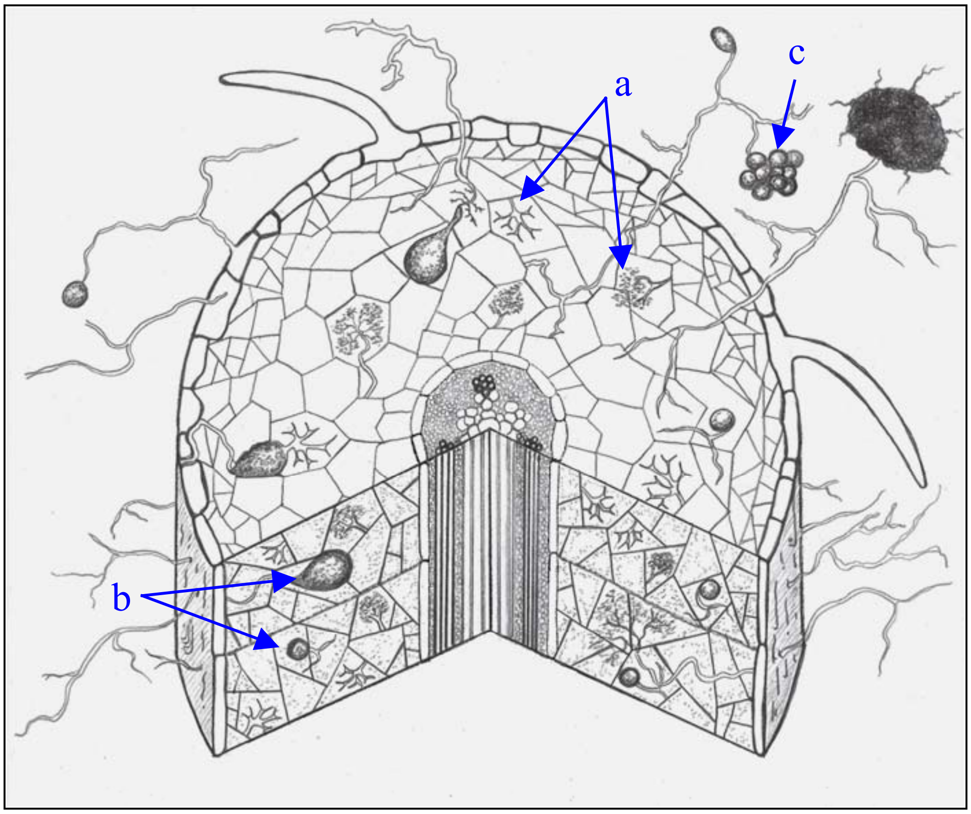

Figure 1. A conceptual radial and cross-section of a root colonized by arbuscular mycorrhizal fungi. a. Arbuscules. b. Vesicles. c. Extra-radical asexual spores. (Drawing by Maria Lee).

In return for the plant-derived organic carbon, phosphorus, nitrogen, and other minerals travel from the fungus to the plant host. Far-reaching extra radical hyphae provide increased mineral uptake for the host. Hyphae are smaller in comparison to most root hairs and therefore gain access to nutrients not ordinarily available to plants. In addition, hyphae are less expensive to produce than root hairs in terms of carbon (Smith and Read 1997). Hattingh et al. (1973) documented the movement of ^{32}P from the soil into *Allium* (onion) plants via symbiotic fungi. To observe the movement of the labeled P from the soil to the plant, a growth chamber was divided into two zones by a fine mesh that excluded roots, but not hyphae, from half of the soil. ^{32}P was added to the soil in the section that excluded roots and the phosphorus was later found in the *Allium*. To demonstrate that the ^{32}P was actually conducted to the plants by the AMF, all hyphal connections were severed. Once the hyphae were cut, no further translocation was detected (Smith and Read 1997). Govindarajulu et al. (2005) showed the transfer of nitrogen from *Glomus intraradices* to *Daucus carota* (common carrot) using a divided petri dish that prevented the movement of nitrogen between the chambers. Nitrogen, in the form of $^{15}NO_3$ or $^{15}NH_4$, was supplied to extra-radical hyphae growing in one half of a divided Petri dish. The nitrogen isotopes were later detected in amino groups within the plant root, which was growing in the other compartment of the Petri dish (Govindarajulu et al. 2005).

Arbuscular mycorrhizal fungi associate with almost all herbaceous plants, making them the type of mycorrhizae most commonly found in prairies. Over the last two decades many studies have demonstrated the important role these soil fungi play in the competition, diversity and productivity of grassland ecosystems (Bever, et al. 2001). Using both greenhouse and field techniques, Van der Heijden et al. (1998) showed that the composition and richness of AMF species can strongly influence plant species composition, diversity, and productivity. Wilson and Hartnett (1998) surveyed the effects of AMF colonization on 95 prairie species grown in a greenhouse. Overall, C_4 grasses and warm-season, perennial forbs showed the greatest increase in biomass production when colonized (Wilson and Hartnett 1998). In a 1999 study, Smith et al. treated field plots with the fungicide benomyl to suppress AM fungi. In benomyl-treated plots, the dominant grass, *Andropogon gerardii* (Big Bluestem), declined while several sub-dominant species increased in cover. In untreated plots, *A. gerardii* cover remained unchanged, suggesting that AM fungi play a role in interspecific competition. Vogelsang et al. (2006) found that certain species of AMF better promoted the productivity of 20-species prairie mesocosms provided with varying quantities and types of phosphorus. In another study looking at AMF and phosphorus, Collins and Foster (2009) used mesocosms planted with prairie species and either treated with AMF or as an untreated control, across a phosphorus gradient. Where phosphorus was limiting, AMF increased diversity and productivity, but where phosphorus levels were high, AM fungi were less influential (Collins and Foster 2009). Even from this small sample of studies, it becomes clear that AM fungi play an important role in the prairie ecosystem. Both the Van der Heijden et al. (1998) and the Vogelsang et al. (2006) studies conclude with the suggestion that loss of AMF species in AMF-dependent ecosystems, such as prairies, could lead to decreased diversity and increased instability.

Thus, the important role arbuscular mycorrhizal fungi play in the prairie ecosystem has been clearly documented (see also Bever et al. 2001). It is also known that some modern agricultural practices, including plowing, disking, and the application of pesticides and other chemicals, may decrease or eradicate AMF populations (Helgason et al. 1998). Since a large number of prairie restorations are located in former agricultural sites, it would be helpful to determine a practical way to include AM fungi when planting prairie plants from seed (Camill et al. 2004).

Several studies have examined the effectiveness of adding arbuscular mycorrhizal fungi to ecosystems to improve the success of restoration projects. Smith et al. (1998) demonstrated that prairie plants growing in restoration plots inoculated with AMF from a local prairie had higher AMF colonization than plants in control plots. Total percent cover of native plants did not differ between treated and control plots, but percent cover of native grasses was higher in the treated plots (Smith et al. 1998). Rowe et al. (2007) assessed the effectiveness of two commercial AMF inocula and an AMF inoculum created from field soil. In a greenhouse, six native montane species and the invasive grass *Bromus tectorum* (cheatgrass) were grown and treated with the three inoculum types alone and in combination. Late-successional species reacted positively to the field soil inoculum while early-successional species and the invasive grass reacted negatively. The commercial inocula resulted in low levels of root colonization by the fungi and were determined to be ineffective at the manufacturer's suggested rate (Rowe et al. 2007). These results are similar to those of Tarbell and Koske (2007), who found that five of the eight tested commercial inocula failed to colonize the roots when applied at the listed rate. Such studies emphasize the importance of testing inoculum application rates prior to large-scale use. Finally, in a 2008 study, White et al. tested a field soil inoculum created from a local prairie and commercial inocula in a roadside prairie restoration. Four treatments and application techniques were used: local inoculum in trenches (to simulate a seed drill), commercial inoculum in trenches, control with trenches, and broadcast local inoculum. Seed was planted at the same time and manner as the inocula. After the first growing season, increased colonization was found in both local and commercial inocula regardless of application technique. However at the end of three growing seasons, there was no significant difference in the rate of prairie establishment between inoculated and control sites (White et al. 2008).

The growing body of research investigating the addition of AMF into prairie restorations shows promise. Several of the above studies utilized inocula synthesized from local sources (Smith et al. 1998; Van der Heijden et al.1998; Wilson and

Hartnett 1998; Vogelsang et al. 2006; Rowe et al. 2007; White et al. 2008). While this technique may yield an appropriate mix of species, it has several potential drawbacks. Disturbance of the donor remnant, cost, the quantity required for larger restorations, and the fact that an appropriate source is not always available are all detractors from using a local source. It would therefore be helpful to determine if commercially offered AMF inocula provide benefits to prairie species. However, the appropriate application rate of commercial inocula remains a question (Rowe et al. 2007; Tarbell and Koske 2007). Additionally commercial inocula have not been tested in a greenhouse study with prairie species grown in unsterilized soil which mimics natural restoration conditions and possibly provides better growing conditions for AMF (Smith and Read 2007).

Research Objectives

The purpose of this study is to determine if two commercially available arbuscular mycorrhizal fungi inocula will increase AMF colonization and plant biomass in five native sand prairie species and the invasive, exotic grass, *Bromus inermis* (Smooth Brome). The sand prairie ecosystem is a geographically limited form of tallgrass prairie that was locally abundant in southwestern Wisconsin prior to European settlement. Sand prairies are of local conservation concern both because they are rare and because of the high number of endemic species (Wisconsin Department of Natural Resources 2005).

Plants will be grown in field-collected soil, mixed with inocula, and grown in a greenhouse for approximately 3 months. Soils will be collected from two sites that typify different pre-restoration conditions. The first collection area will be located within the New Amsterdam Grassland, which is owned and managed by the Mississippi Valley Conservancy. The collection site will be a meadow dominated by *Bromus inermis* that was planted when the site was enrolled in the Conservation Reserve Program (CRP) at least 20 years ago (Jessica Bolwahn, *personal comm.*). The CRP is designed to help farmers protect natural resources and reduce soil loss on highly erodible land. In prairie areas, this often includes planting a permanent cover of non-native grasses such as *B. inermis*. Thus, the soil from this site will be representative of conditions found when prairie restorations are installed into pastures, meadows, or fallow ground. The second soil source will be the Dummer Farm, which is currently used for corn and soybean production (*personal observation*). The soil from this site will be used because it accurately represents site conditions found in currently tilled ground.

The field soils will be mixed with commercially produced AMF inocula. One inoculum will be a low-diversity mix containing three common *Glomus* species. The other inoculum will provide a more diverse set of seven AMF species. The two inocula will be combined with both soil types at the manufacturer's suggested levels. In addition, due to the fact that previous research has sometimes found these levels insufficient, both inocula will also be evaluated at twice the recommended amounts (Tarbell and Koske 2007). An untreated control of each soil type will also be tested to determine whether AMF are naturally present in either field site.

Four native sand prairie species and a non-native, invasive grass will be grown in the inoculated and control soils. Two native grasses will be included (both C_4 and C_3 grass species). In addition, two common sand prairie forbs will be grown as examples of early and late successional forbs. Finally, *Bromus inermis* will be examined to determine if the addition of AMF affects this common sand prairie invader. Percent colonization of the roots and biomass of the plants will be evaluated to determine the effects of inoculum type and application rate across plant species and soil type.

CHAPTER II

CAN COMMERCIAL AMF INOCULUM IMPROVE PRAIRIE RESTORATION? COLONIZATION AND GROWTH EFFECTS OF INOCULUM OF FUNGI ON SAND PRAIRIE PLANTS AND SMOOTH BROME IN FIELD SOILS.

Introduction

Prior to European settlement of North America, prairies covered roughly 975 million acres in the heart of the continent (Lemon 1968). It is estimated that only one hundredth of one percent of the original tallgrass prairie remains, most having been converted to corn and soybean production (Smith 1990; Neely and Heister 1987). As a result, North American prairies are one the most endangered ecosystems in North America and in the world (Samson and Knopf 1994).

In response to the rapid loss of the prairie ecosystem, many individuals and organizations have worked to preserve and restore remaining prairie parcels. Prairie restoration requires careful planning and implementation of a combination of techniques, with the ultimate goal of producing an ecosystem that resembles a natural prairie in terms of structure and function (Kline 1997; Schramm 1990). In natural prairies, that structure and function are maintained by forces that work together like pieces in a jigsaw puzzle, simultaneously influencing the effects of multiple factors. Just like a puzzle, a restoration that is missing pieces will not closely resemble a natural prairie. Most restoration techniques focus on the establishment and performance of plant species. One piece of this restoration "puzzle" that is often overlooked is the role played by mycorrhizal fungi.

Mycorrhizal fungi are a diverse group of fungi that form mutualistic symbiotic relationships with plants. The fungi produce a network of hyphae that extend beyond the rhizosphere of the host plant and assist with water and nutrient uptake, especially phosphorus and nitrogen. In turn, the mycorrhizal fungus is supplied with photosynthate in the form of sugar (Smith and Read 1997). Arbuscular mycorrhizal fungi (AMF, Phylum Glomeromycota), also known as endomycorrhizal fungi and vesicular-arbuscular mycorrhizal (VAM) fungi, are the most common form of mycorrhizal symbiosis with an estimated 200,000 host plants (Smith and Read 1997; Helgason et al. 2007). Associating with almost all herbaceous plants, AMF are the type of mycorrhizae most commonly found in prairies. Dark septate endophytes (DSE) are a diverse group of non-pathogenic, root-colonizing fungi that are found in a wide variety of plant hosts and habitats, including prairies (Jumpponen and Trappe 1998; Mandyam and Jumpponen 2008). DSE are known to co-exist with AMF, but in comparison to AMF, the role of DSE symbiosis is poorly understood (Mandyam and Jumpponen 2005).

Over the last two decades many studies have demonstrated the important role AMF play in the competition, diversity and productivity of grassland ecosystems (e.g. Van der Heijden et al. 1998; Wilson and Hartnett 1998; Bever, et al. 2001; Vogelsang et al. 2006; Collins and Foster 2009). It is also known that some modern agricultural practices, including plowing, disking, and the application of pesticides and other chemicals, may decrease or eradicate AMF populations (Helgason et al. 1998). Since a large number of prairie restorations are located in former agricultural sites, it would be helpful to determine a practical way to include AM fungi when planting prairie plants from seed (Camill et al. 2004).

Several studies have examined the effectiveness of various methods of adding arbuscular mycorrhizal fungi to ecosystems to improve the success of restoration projects. Smith et al. (1998) demonstrated that prairie plants grown in plots inoculated with AMF reproduced from a local prairie had higher AMF colonization than plants in control plots. Total percent cover of native plants did not differ between treated and control plots, but percent cover of native grasses was higher in the treated plots (Smith et al. 1998). White et al. (2008) tested a field soil inoculum created from a local prairie and commercial inocula in a roadside prairie restoration. Increased AMF colonization was observed in all inoculated plots relative to controls, although this difference disappeared by the end of the third growing season (White et al. 2008). Rowe et al. (2007) assessed the effectiveness of two commercial AMF inocula and field soil as an AMF inoculum. In a greenhouse, six native montane species and the invasive grass *Bromus tectorum* (cheatgrass) were grown and treated with the three inoculum types alone and in combination. Late-successional species reacted positively to the field soil inoculum while early-successional species and the invasive grass reacted negatively. The commercial inocula resulted in low levels of root colonization by the fungi and were determined to be ineffective at the manufacturer's suggested rate (Rowe et al. 2007). Similarly, Tarbell and Koske (2007) found that five of eight tested commercial inocula failed to colonize the roots when applied at the listed rate. Such studies emphasize the importance of testing inoculum application rates prior to large-scale use.

The studies above are a sample of the growing body of research investigating the use of AMF in prairie restoration. Several of the works utilized inocula propagated from local sources (Smith et al. 1998; Rowe et al. 2007; White et al. 2008). While this technique may yield an appropriate mix of species, it has several potential drawbacks, including disturbance of the donor remnant, cost, the quantity required for larger restorations, and the fact that an appropriate source is not always available.

An alternative is the use of commercially available inoculum. Although the number of species of AMF represented in these inocula is not diverse, most of the species have a wide host range, colonize plants from spores successfully, and would be expected to colonize plants in a restoration (Klironomos and Hart 2002). Commercial inocula have not been tested in a greenhouse study with prairie species grown in unsterilized soil. Field soil would better mimic natural restoration conditions and possibly provides better growing conditions for AMF (Smith and Read 2007). For restoration sites that have heavily disturbed soils, such as agricultural fields, commercial inoculum could provide an inexpensive and simple way to reintroduce AMF. However, the appropriate application rate of commercial inocula remains a question (Rowe et al. 2007; Tarbell and Koske 2007).

The purpose of this study was to determine if two commercially available arbuscular mycorrhizal fungi inocula would increase AMF colonization and plant biomass in four native sand prairie species and the invasive, exotic grass, *Bromus inermis* (Smooth brome). Furthermore, the effectiveness of the commercial inocula was examined at the manufacturer’s recommended application rate and at double strength. Finally, plant and fungal responses to inocula were evaluated in soils from two contrasting potential restoration sites: a current agricultural field and an area enrolled in the CRP and planted with Smooth brome more than 20 years ago. The Conservation Reserve Program (CRP) is designed to help farmers protect natural resources and reduce soil loss on highly erodible land. In prairie areas, this often includes planting a permanent cover of non-native grasses such as *B. inermis*, which can present a problem to the restoration of these sites (Bolwahn 2010). Working in the greenhouse allowed for the examination of plant responses to treatments in a controlled environment and to determine total plant biomass at harvest.

Methods

Site Information and Soil Collection

Soil for the experiment was collected from two sites. According to USDA soil survey maps Chelsea Fine Sand is the dominant soil type for both sites, which has 93.2% sand content (Soil Survey Staff 2011). The sites are located 2 km apart, have a continental climate and receive an average of 81 cm of rain annually. Average high temperatures range from -4° C in January to 29° C in July. Low temperatures average between -14° C in January and 17° C in July (National Weather Service 2009). Both sites are candidates for restoration into sand prairie, a geographically limited form of tallgrass prairie that was locally abundant in southwestern Wisconsin prior to European settlement. Sand prairies are of local conservation concern both because they are rare and because of the high number of endemic species (Wisconsin Department of Natural Resources 2005).

On 12 November 2008, soil was gathered at the New Amsterdam Grassland, which is owned and managed by the Mississippi Valley Conservancy. The collection site is a meadow dominated by *Bromus inermis* that was planted when the site was enrolled in the CRP at least 20 years ago (Jessica Bolwahn, *personal comm*.). Thus, the soil from this site is representative of conditions found when prairie restorations are installed into pastures, meadows, or fallow ground. Soil collected from this site is hereafter referred to as “CRP soil.”

On 1 December 2008, soil was collected from the Dummer Farm located approximately 2 km south of the New Amsterdam Grassland site. This collection site is an agricultural field that had been planted in soybeans, *Glycine max* (Linn.) Merr., in 2008 and corn, *Zea mays* L., in 2007. The soil from this site was used because it accurately represents site conditions found in currently tilled ground. Snow fell the night prior to gathering the soil, but the ground under the snow was still unfrozen. Snow was removed from collection site prior to digging. Soil collected at this site is referred to as “tilled soil.”

At both sites, soil was dug by hand from several arbitrarily chosen locations. Each hole was approximately 15 cm deep and 30 cm in diameter. Large roots, rhizomes, and leaf material were manually removed, and the soil was placed in plastic tubs. The tubs were stored at ambient outdoor temperature until a week before planting.

Soil Preparation

One week prior to seeding, soil was moved to the Cowley Hall Greenhouse at the University of Wisconsin-La Crosse and allowed to warm to room temperature (around 26°C). To mimic natural prairie restoration soil conditions, field-collected soil was not sterilized prior to planting. The soil was sifted through a ¼ inch mesh to remove remaining vegetative material and to homogenize the soil. Both soil types (CRP soil and tilled soil) were divided into 5 equal portions to be mixed with one of four AMF treatments or left as a control. The AMF treatments utilized two commercial AMF inocula: a three-species *Glomus* inoculum reported to contain *Glomus aggregatum,* *Glomus intraradices,* and *Glomus mosseae* (Garden-Ville Mycorrhizal Fungi, produced by Garden-Ville, Austin, Texas, www.garden-ville.com) and an eight-species AMF mix reported to contain *Gigaspora margarita, Glomus aggregatum, Glomus clarum, Glomus deserticola, Glomus intraradices, Glomus monosporus, Glomus mosseae,* and *Paraglomus brasilianum* (Endomycorrhizal Inoculant (BEI) by Bio Organics™, Santa Maria, California, www.bio-organics.com). The four AMF treatments included each inoculum type at the product's recommended application rate per cubic yard and at twice the recommended rate (Table 1). Thus, the experiment included 10 total soil type and AMF treatment combinations ("soil treatments" hereafter, Table 2). The appropriate weight of inocula was measured out and thoroughly mixed into the soil by hand (Table 1). Hereafter the three species Glomus mix at the manufacturer's suggested rate and at the doubled rate will be referred to as GL1 and GL2 respectively. Likewise, the eight species mix at the recommended and double rates will be referred to as Mix1 and Mix2.

Table 1. Quantity of AMF inoculum added to 0.05 yd^3 (0.038m^3) of soil to establish AMF treatments in both CRP and tilled soils.

AMF Treatment	Rate	Manufacturer's recommended rate per yd^3	Amount of inoculum added (g) per 0.05 yd^3 (0.038m^3)
3 species *Glomus* – GL1	1x	2 Lbs	11.34
3 species *Glomus* – GL2	2x		22.68
8 species mix – Mix1	1x	1 Lb	5.67
8 species mix – Mix2	2x		11.34

Table 2. Composition of the ten AMF treatments and soil type combinations (soil treatments) used in the experiment. Each of the five plant species were planted into ten replicate tubes per soil treatment (Figure 2).

Tube Number	AMF Treatment	Soil Type
1-10	control	CRP
11-20	GL1	CRP
21-30	GL2	CRP
31-40	Mix1	CRP
41- 50	Mix2	CRP
51-60	control	Tilled
61-70	GL1	Tilled
71-80	GL2	Tilled
81-90	Mix1	Tilled
91-100	Mix2	Tilled

Each soil treatment type was used to fill 50 clean 9-mL Fir Cell Cone-tainers™ (Stuewe and Sons, Corvallis, OR), hereafter referred to as tubes. To keep the tubes together and upright, five filled tubes were placed into each of 10, clear plastic deli containers, one-quart size (Figure 2). All the tubes in a given deli container were filled with the same soil treatment, eliminating the issue of cross contamination of soil treatments from splash over during watering.

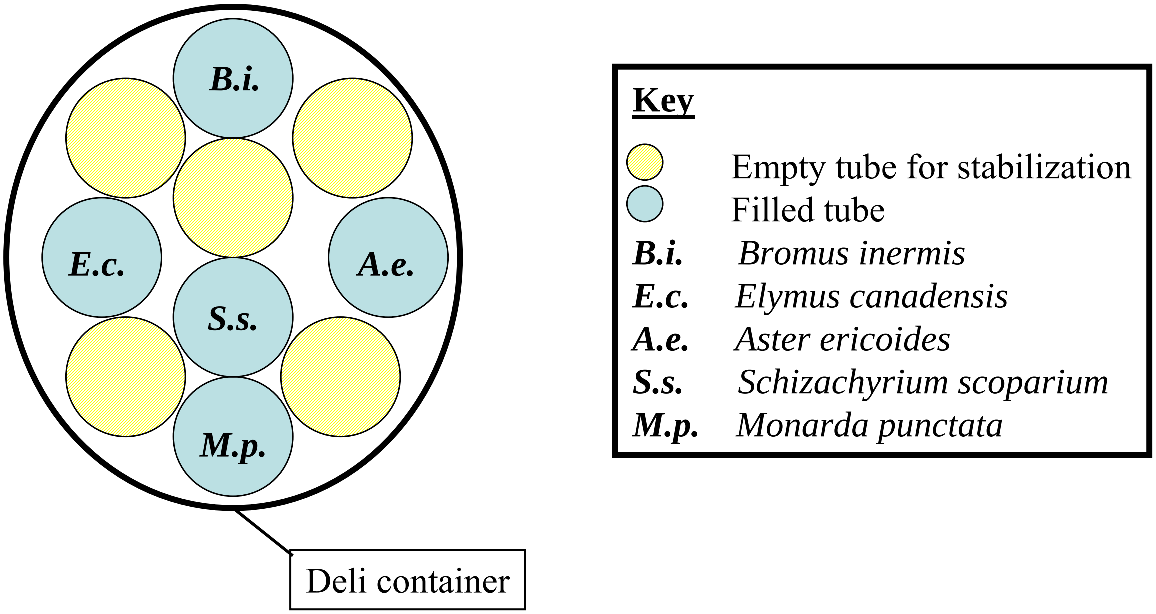

Figure 2. Schematic diagram of the arrangement of tubes in deli containers. All the filled tubes in a given container contained the same soil treatment, and ten deli containers were created per soil treatment (*N* = 10 for each plant x soil treatment combination).

Planting

Five perennial plant species were chosen for the experiments (Table 3). These included a non-native invasive grass *Bromus inermis* Leyss. (Smooth brome), a C_3 species, along with two prairie grasses *Elymus canadensis* L. (Canada Wild Rye), also a C_3 species, and *Schizachyrium scoparium* Michx. (Little Bluestem), a C_4 species. Two common sand prairie forbs were grown: *Aster ericoides* L. (Heath Aster), and *Monarda punctata* L. (Spotted Beebalm). These species are examples of late- and early-successional forbs, respectively. The native species are commonly planted in sand prairie restorations. Seeds were purchased from Prairie Moon Nursery in Winona, Minnesota and were not pretreated. Each plant species was seeded into one filled tube per deli container, and thus grown in each soil treatment in 10 independent replicates (a total of 500 plants in the entire experiment, Figure 2). All plants were seeded at a rate of approximately five seeds per tube and watered as needed throughout the course of the experiment. Tubes were periodically checked and thinned to contain only one individual. Containers were randomly placed on the greenhouse benches. The maximum daytime temperature in the greenhouse ranged from near 26°C at the beginning of the experiment to around 38°C by the end.

Table 3. Plant species included in these experiments. Species were chosen to represent a diversity of growth forms and physiological strategies.

Scientific Name	Common Name	Growth Form	Native/ Non-Native
Bromus inermis	Smooth Brome	C_3 grass	Non-native
Elymus canadensis	Canada Wild Rye	C_3 grass	Native
Schizachyrium scoparium	Little Bluestem	C_4 grass	Native
Aster ericoides	Heath Aster	Late successional forb	Native
Monarda punctata	Spotted Beebalm	Early successional forb	Native

Harvest

Plants were harvested after 83 days of growth. Survivorship for each species was determined by counting the total number of tubes in which a living individual was present on the harvest date. Since multiple seeds were planted per tube, each “non-survivor” represents the failure to germinate or death of at least five individuals. Aboveground biomass was removed, dried to constant mass at 60°C, and weighed. Belowground biomass was removed from the soil and rinsed clean in water. Cleaned roots were immediately preserved in a 50% ethanol solution. Total wet mass of each root system was measured, and a small subsample was removed, weighed, and returned to the ethanol solution to be used later to determine percent colonization. The remaining roots were placed in coin envelopes, dried to constant mass, and weighed. Total belowground biomass was calculated by using the ratio of the total and partial wet biomasses to the partial dry biomass and solving for the total dry biomass.

Percent Colonization

Fungal hyphae in preserved roots were stained using Shaeffer Black ink and household vinegar (5% acetic acid) (Vierheilig et al. 1998). After staining, the roots were rinsed and destained in tap water for 20 minutes and stored at room temperature in tap water. Roots were scored for percent colonization using the magnified intersection method described by McGonigle et al. (1990). For each individual root system, three 1 cm segments were examined for fungal colonization by making five perpendicular passes through each of the three segments. Hyphae, arbuscules, and vesicles that intercepted the vertical crosshair were recorded. All other fungi observed in the roots, such as dark septate endophytes, were also noted. For the grass species, seven randomly chosen individuals within each of the ten soil treatments were scored for percent colonization. Due to lower survivorship in *Aster*, five individuals per treatment group were scored. If there were fewer than seven/five root samples available in a given treatment, additional samples were scored from randomly selected individuals in that treatment (maximum three re-sampled individuals for treatments involving *Bromus*, zero for *Elymus*, 13 for *Schizachyrium* and three for *Aster*).

Statistical Analyses

Chi-square analysis was used to compare the number of surviving seedlings of each species in CRP vs. tilled soil. Two-way ANOVAs were used to examine the interactive effects of soil type and AMF treatment on total plant biomass, root to shoot ratio, and percent colonization of AMF. These analyses were performed separately for each plant species. Only plants selected for percent colonization sampling (see above) were included in biomass analyses. Two-way ANOVA was also used to examine the effects of soil type, plant species, and their interaction on percent colonization of the dark-septate endophytes (DSE). All data were examined for normality of distribution and equality of variances and transformed as necessary to meet the assumptions of ANOVA. When soil type was significant, Student's t-tests were used to compare the two means. If AMF treatment or species was significant, Tukey-Kramer HSD tests were used to compare the mean values. Finally, linear regression was used to examine the relationship between percent AMF colonization in CRP soil and total biomass for each species. Analyses were conducted using JMP® 8 (SAS Institute Inc., Cary, NC).

Results

Seedling Survivorship

Chi-square analysis indicated that seedling survivorship was significantly higher in the CRP soil for *Bromus*, *Elymus*, *Schizachyrium*, and *Monarda*, but not for *Aster* (Table 4). The appearance of *Monarda* seedlings was consistent with the symptoms of damping off, but this was not the case for the grasses (*personal observation*). Due to extremely low survivorship of the *Monarda* individuals, they were not included in any further analysis.

Table 4. Chi square results for seedling survivorship (number of tubes containing a living individual at harvest) in CRP vs. tilled soil (df = 1). Possible number of survivors per soil type is 50.

Species	Number of survivors		χ^2	*P*
	CRP	Tilled		
Bromus inermis	48	42	4.0	0.046
Elymus canadensis	50	46	4.2	0.041
Schizachyrium scoparium	44	24	18.4	< 0.001
Aster ericoides	36	30	1.6	0.206
Monarda punctata	16	4	9.0	0.003

Plant Responses

The effect of soil type and inoculum treatment on total dried biomass at harvest varied among the plant species. Neither soil type or inoculum treatment had an effect on the total biomass for *Bromus* (Table 5, Figure 3a). In contrast, soil type significantly affected both *Elymus* and *Schizachyrium* biomass (Table 5, Figures 3b and c). *Elymus* plants grown in CRP soil were 44% larger than those grown in tilled soil (Figure 3b). Likewise, *Schizachyrium* plants propagated in CRP soil had more than double the biomass (130% larger) than their counterparts in tilled soil. For *Aster*, soil type had no effect on plant biomass but inoculum type did (Table 5, Figure 3d). The greatest biomass was found in treatments GL1, GL2 and Mix2. Conversely Mix1 had the lowest biomass (one quarter of the combined mean biomass of plants grown in the GL1, GL2 and Mix2 treatments). The mean biomass of the *Aster* control group was intermediate (approximately half the mean biomass of plants grown in GL1, GL2, or Mix2).

Table 5. Results of two-way ANOVA testing the effect of soil type, inoculum treatments, and their interaction on total biomass of *Bromus*, *Elymus*, *Schizachyrium,* and *Aster* plants.

	Bromus			*Elymus*			*Schizachyrium*			*Aster*		
Treatment	df	F	*P*	df	F	*P*	df	F	*P*	df	F	*P*
Soil	1, 57	0.10	0.748	1, 60	10.14	0.002	1, 47	7.77	0.008	1, 37	1.70	0.200
Inoculum	4, 57	0.82	0.516	4, 60	0.16	0.156	4, 47	0.62	0.649	4, 37	4.27	0.006
Interaction	4, 57	0.67	0.618	4, 60	0.63	0.642	4, 47	0.21	0.929	4, 37	0.87	0.491

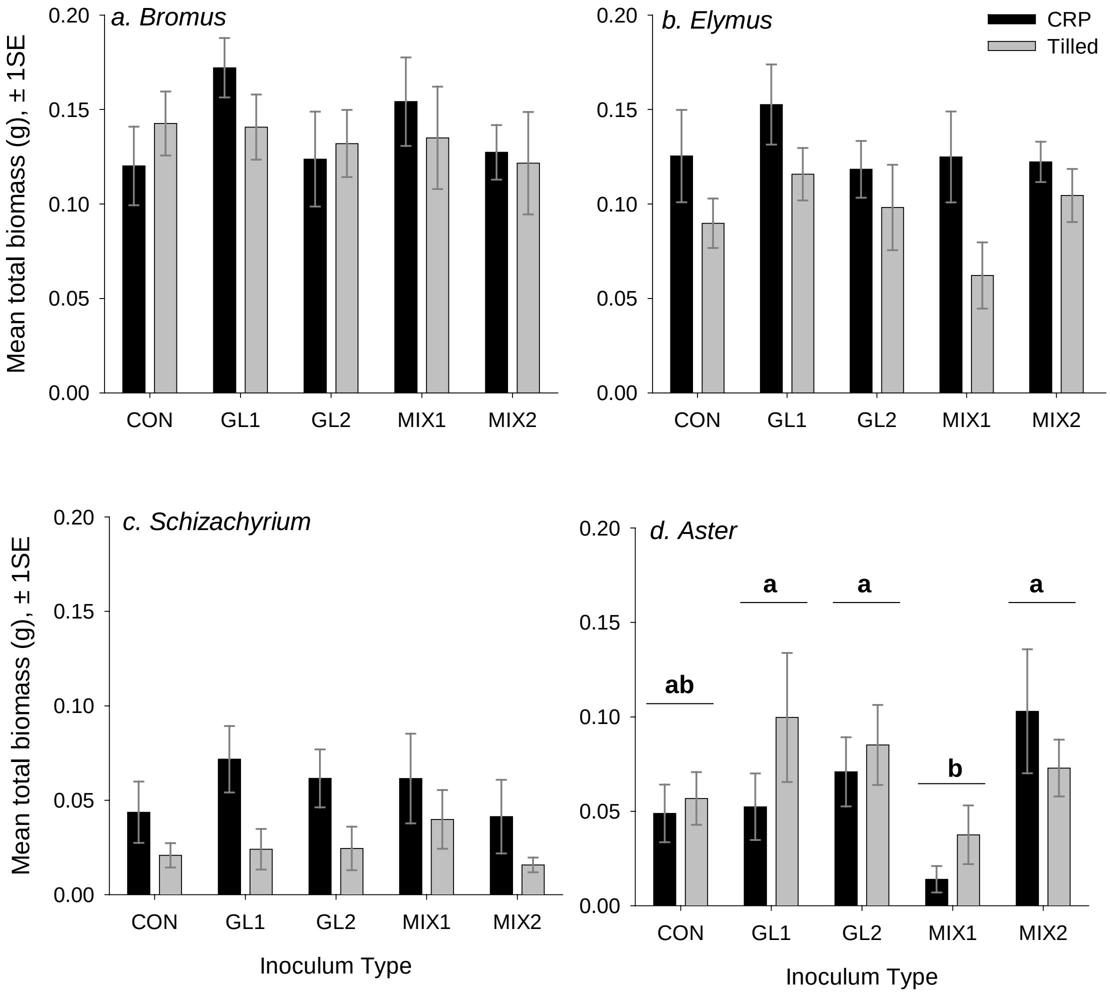

Figure 3. Mean total dry biomass at time of harvest for *Bromus*, *Elymus*, *Schizachyrium*, and *Aster*. Black bars represent plants grown in CRP soil and gray bars those grown in tilled soil. Letters represent inoculum treatments that are significantly different in Tukey post-hoc means comparisons. For *Elymus* and *Schizachyrium*, biomass of plants grown in tilled soil was significantly less than those grown in CRP soil (see Table 5 for ANOVA statistics).

Fungal Responses

The effect of soil type and inoculum treatment on percent colonization by arbuscular mycorrhizal fungi varied among the four plant species. Colonization by AMF was observed in each plant species (Figure 4). Soil type had a significant effect on colonization for all three grasses (Table 6, Figure 5a, b, and c). Mean AMF colonization in CRP soil was 8, 13, and 2 times greater in *Bromus*, *Elymus*, and *Schizachyrium*, respectively, when compared to individuals in tilled soil. As with total biomass, inoculum treatment, but not soil type, had a significant effect on percent colonization in *Aster* (Table 6, Figure 5d). However, pairwise comparisons of percent colonization in the individual inoculum treatments were not significant.

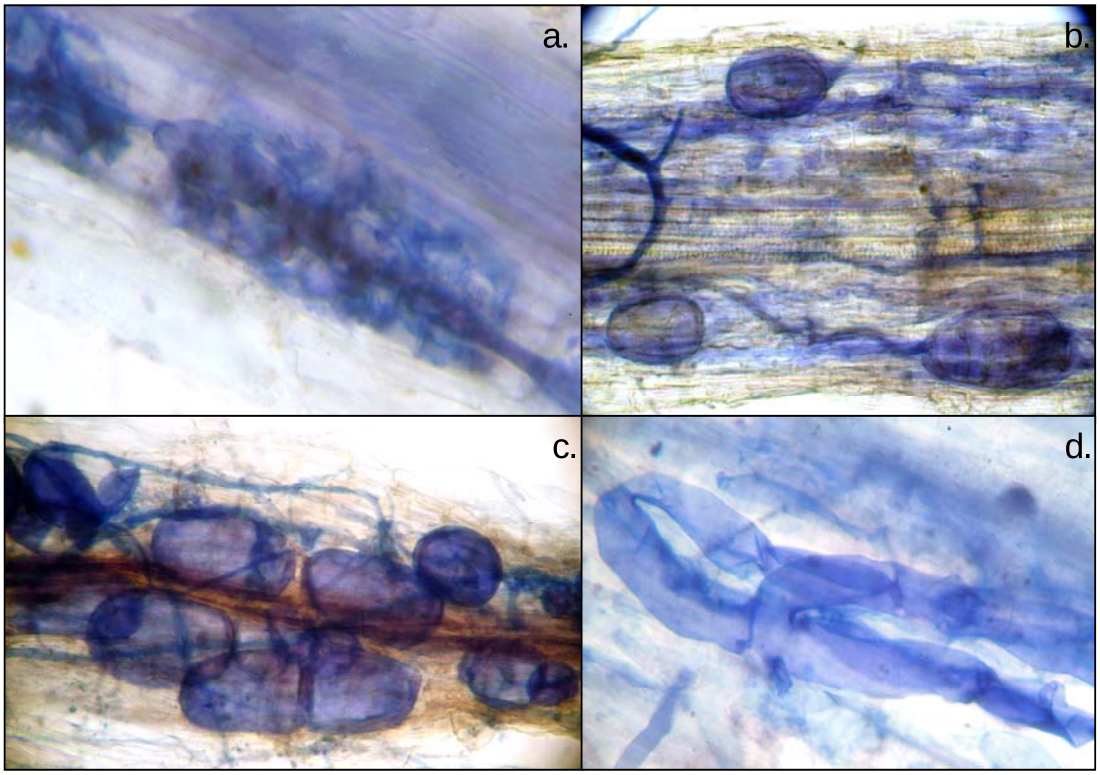

Figure 4. Arbuscular mycorrhizal fungal structures observed in experimental plants. a. Arbuscules in *Elymus* (100x) b. Vesicles in *Aster* (40x) c. Vesicles in *Bromus* (40x) d. Hyphal coil in *Bromus* (100x).

Table 6. Results of two-way ANOVA testing the effect of soil type, inoculum treatments, and their interaction on percent colonization of arbuscular mycorrhizal fungi in *Bromus*, *Elymus*, *Schizachyrium,* and *Aster* plants.

		Bromus		*Elymus*		*Schizachyrium*		*Aster*	
Treatment	df	F	*P*	F	*P*	F	*P*	F	*P*
Soil	1, 60	27.96	< 0.001	61.72	< 0.001	16.67	< 0.001	1.16	0.298
Inoculum	4, 60	0.60	0.667	0.70	0.596	0.93	0.450	3.02	0.029
Interaction	4, 60	0.97	0.430	2.10	0.092	0.45	0.775	0.71	0.590

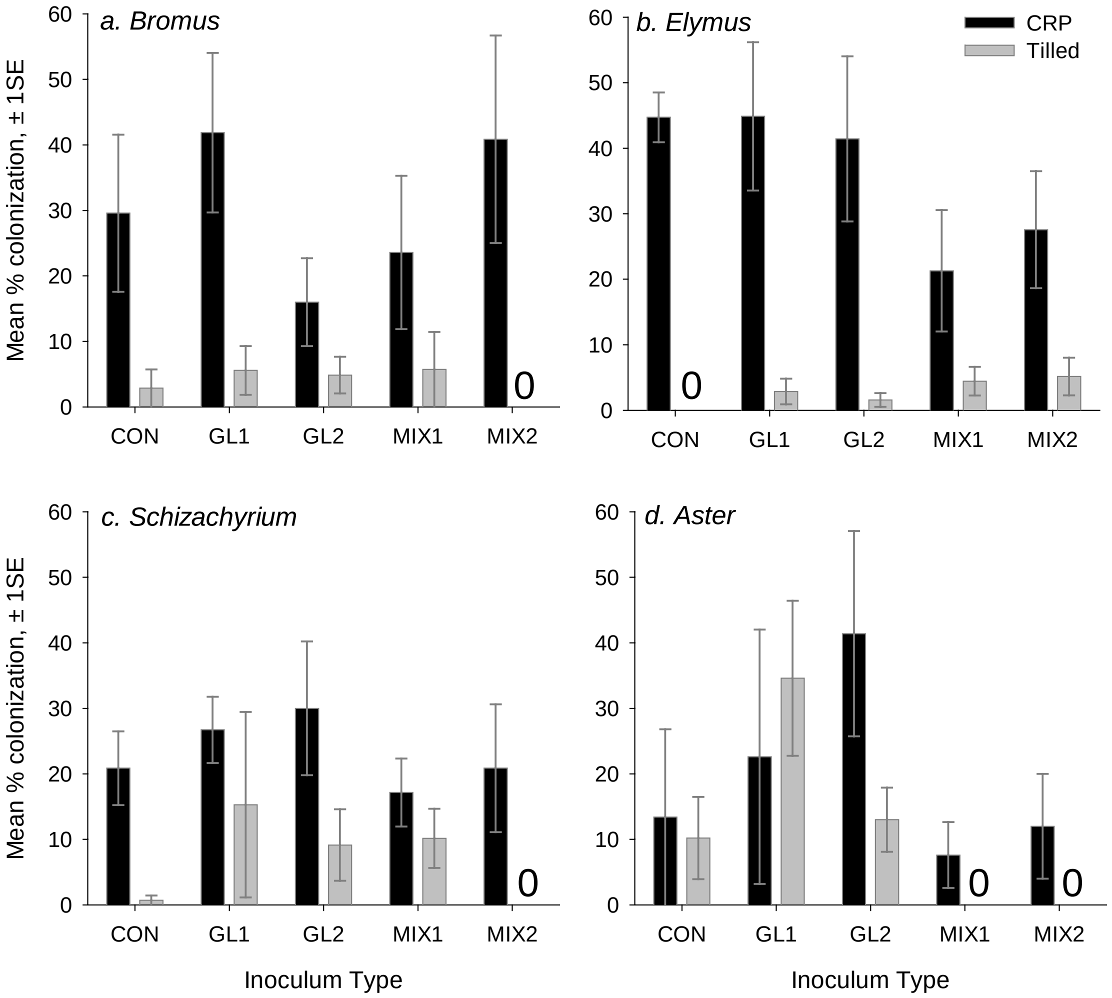

Figure 5. Mean percent colonization by arbuscular mycorrhizal fungi for *Bromus*, *Elymus*, *Schizachyrium*, and *Aster*. Black bars represent percent colonization of plants grown in CRP soil and gray bars show percent colonization in tilled soil. For the three grasses, colonization of plants grown in CRP soil was significantly greater than those grown in tilled soil. Inoculum treatment significantly influenced percent colonization in *Aster* (see Table 6 for ANOVA statistics).

The relationship between percent AMF colonization in CRP soils (where there was an appreciable level of AMF colonization) and total biomass at harvest differed between the grasses and *Aster* (Table 7, Figure 6). In *Bromus*, *Elymus*, and *Schizachyrium*, percent AMF colonization did not affect biomass. However there was a significant positive relationship between colonization and biomass for *Aster*.

Table 7. Regression statistics for the relationship between percent colonization of AMF and total dry biomass at harvest for plants grown in CRP soil.

Species	df	R^2	F	*P*
Bromus inermis	1, 32	0.10	3.73	0.062
Elymus canadensis	1, 34	0.05	1.77	0.192
Schizachyrium scoparium	1, 31	0.02	0.46	0.503
Aster ericoides	1, 22	0.24	7.03	0.015

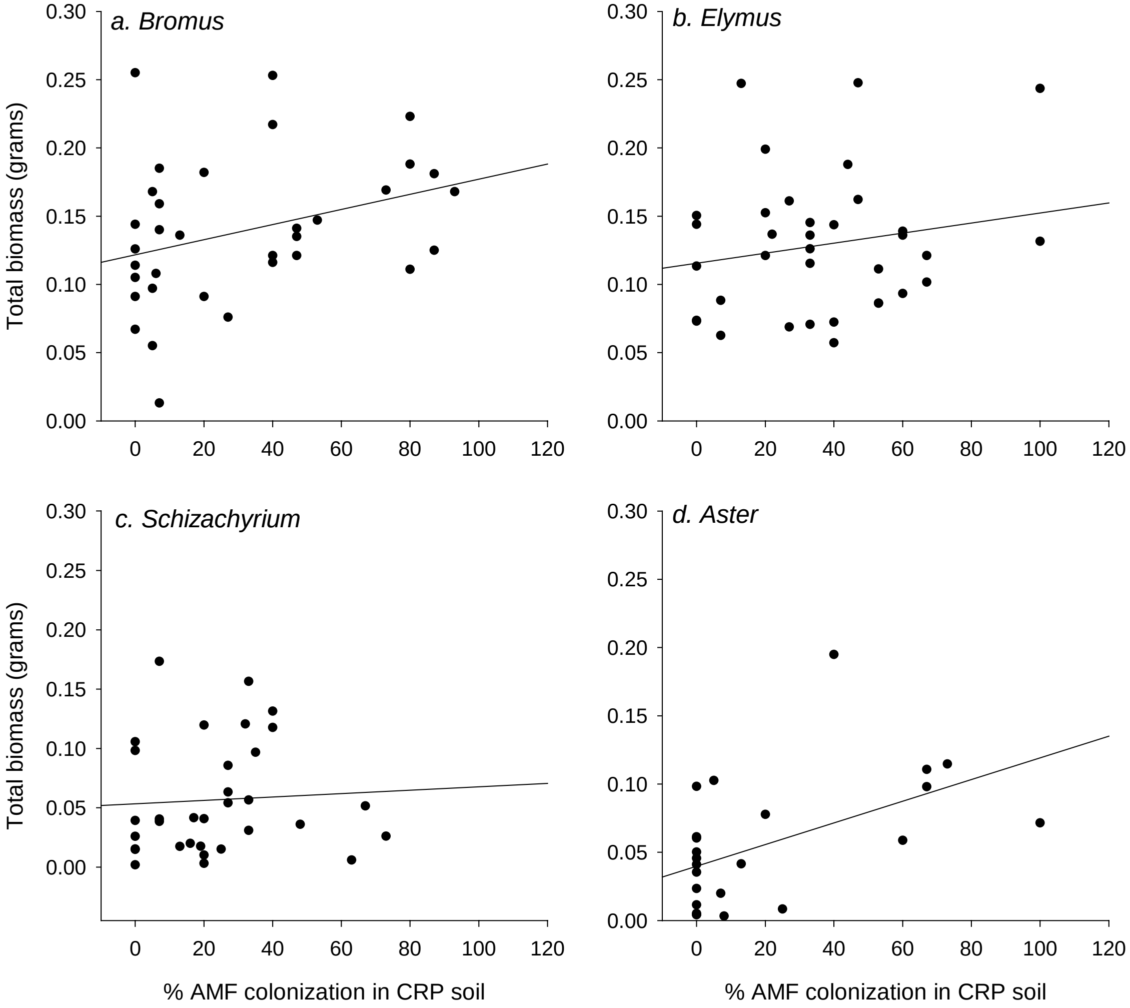

Figure 6. Relationship between percent AMF colonization and total biomass at harvest in CRP soil for *Bromus*, *Elymus*, *Schizachyrium*, and *Aster*, which was significant only for *Aster* (see Table 7 for regression statistics).

Because *Aster* was the only species that appeared to respond significantly to AMF colonization, linear regression was used to examine the relationship between percent AMF colonization and *Aster* belowground versus aboveground allocation (root/shoot ratio). Regardless of soil type, *Aster* plants with the most colonization corresponded to the highest root/shoot ratios (Figure 7, $R^2 = 0.27$, $F_{1,44} = 16.55$, $P < 0.001$).

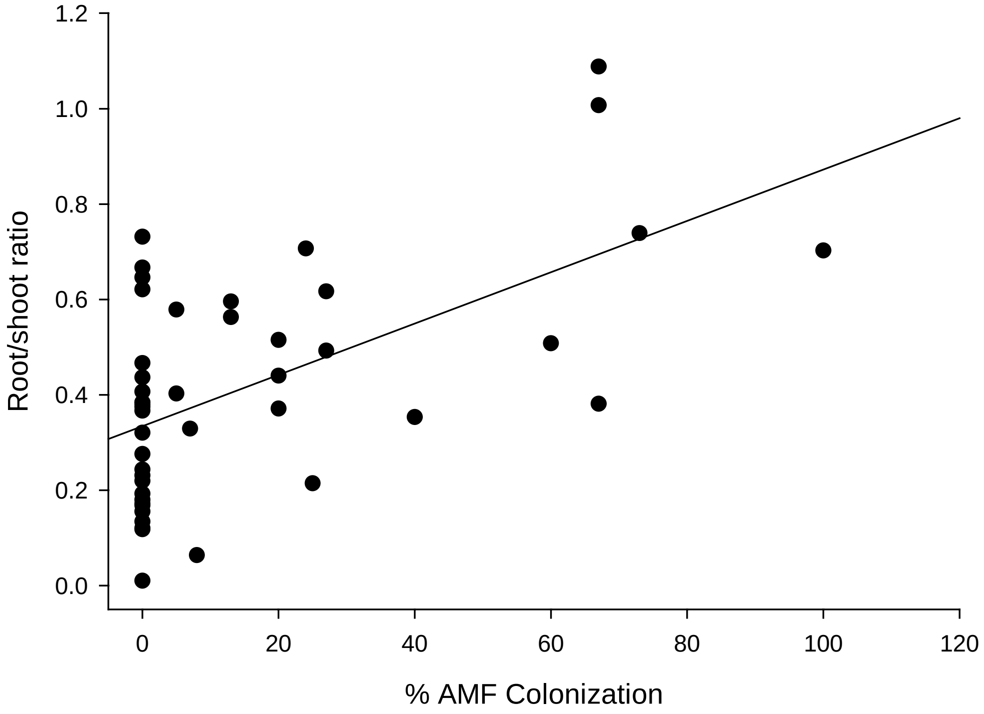

Figure 7. Relationship between percent AMF colonization and root/shoot ratio in *Aster*. See text for regression statistics.

Dark septate endophytes (DSE) were observed in the form of hyphae and microsclerotia in the roots samples of *Bromus*, *Elymus*, *Schizachyrium*, and *Aster* (Figure 8). Percent colonization of DSE was significantly affected by species and soil type (Table 8, Figure 9). The mean percent DSE colonization of the three grass species was four times greater than colonization in *Aste*r. Plants grown in tilled soil had a mean percent DSE colonization was 1.75 times greater than plants raised in CRP soil. The interaction of soil and plant species did not affect the percent colonization of dark septate endophytes.

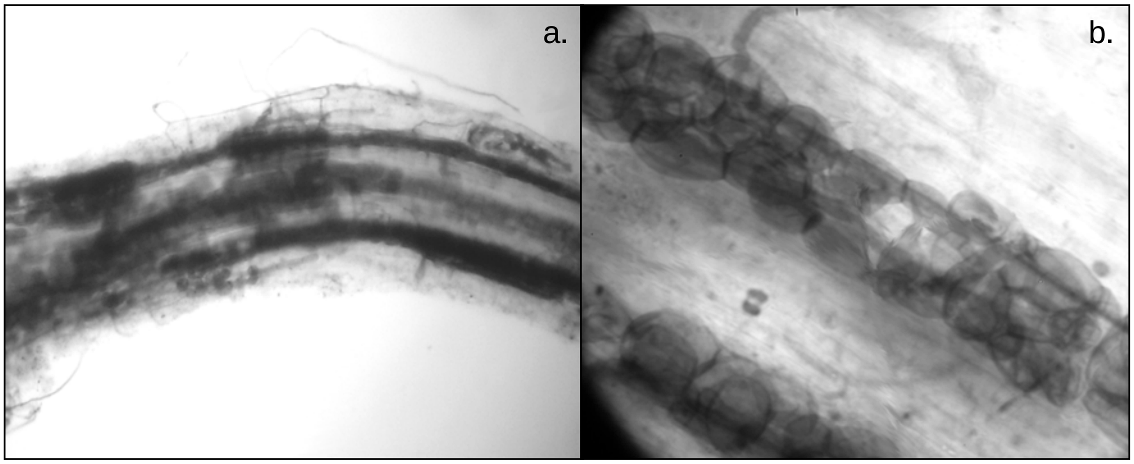

Figure 8. DSE in *Schizachyrium* roots. a. Highly colonized root (40x) b. microsclerotia (100x). Similar structures were observed in all plant species.

Table 8. Results of two-way ANOVA testing the effect of soil type, plant species, and their interactions on percent colonization of dark septate endophytes in experimental plants.

Treatment	df	F	*P*
Soil	1, 252	6.58	0.011
Species	3, 252	7.30	<0.001
Interaction	3, 252	0.88	0.455

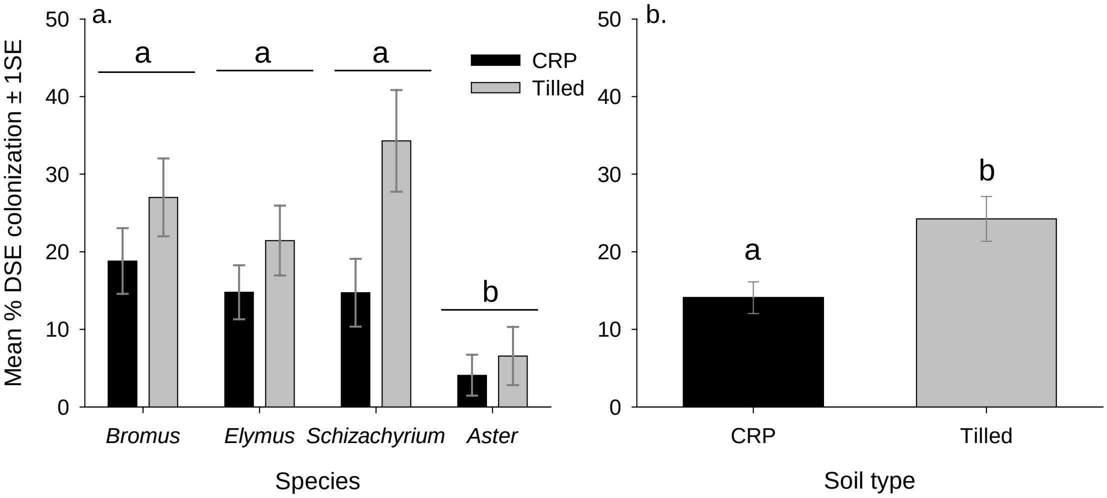

Figure 9. Mean percent colonization by dark septate endophytes for *Bromus*, *Elymus*, *Schizachyrium*, and *Aster*. Black bars represent DSE colonization of plants grown in CRP soil and gray bars that for plants grown in tilled soil. a. Letters represent percent colonization in plant species that were significantly different in Tukey post-hoc means comparisons. b. Across all species DSE colonization was significantly greater for plants grown in tilled soil than in those in CRP soil (see Table 8 for ANOVA statistics).

Discussion

Inoculum Response

Given the important role arbuscular mycorrhizal fungi play in natural prairies, restoration ecologists have long recognized that the addition of AMF to restorations might be a way to increase their likelihood of success. Here, however, the addition of commercial mycorrhizal inoculum had no significant effect on biomass or percent colonization of the exotic grass, *Bromus inermis*, or the native prairie grasses, *Elymus canadensis* and *Schizachyrium scoparium*. From a restoration standpoint, it is encouraging to know that the addition of these inocula does not make *Bromus* more competitive. However, it does not appear to encourage colonization or growth in the native grasses either. If the purpose of the addition of AMF inoculum to a restoration planting is to increase native grass establishment, then these data to not indicate any benefit. The results here agree with another study that examined the benefit of commercial inocula and found the products to be ineffective at increasing AMF colonization (Rowe et al. 2007).

The lack of growth response to mycorrhizal colonization in *Bromus* and *Elymus*, is typical of C3 grasses. In contrast, C4 species are typically regarded as strongly myco-dependant (Wilson and Hartnett 1998). The lack of response in *Schizachyrium* in this study however, parallel those of several other studies conducted in unsterilized or sandy field soils in which this C4 species displayed no growth response to the presence of AMF (Seo et al. 1988; Meredith and Anderson 1992; Anderson and Roberts 1993).

As AMF colonization was observed in control groups for both soil types, it is seems likely that most of the colonization observed in all of the individuals was from ambient soil fungi. Although, without molecular testing, it cannot be determined if the observed colonization in the treated individuals is from the soil or the inocula. Even if there was a shift of AMF species due to the inocula, it did not change the lack of mycorrhizal responsiveness in the grasses. Inocula could be tested in sterile soil, but the sterilization process alters the soil composition in terms nutrient availability and microbe community so that results are no longer comparable (Anderson and Roberts 1993; Anderson et al. 1994; Wilson and Hartnett 1998). Moreover, the colonization results obtained from sterilized soil would not be applicable to the field soil conditions found in prairie restorations.

Unlike the three grasses species, plant productivity and AMF colonization in *Aster* was significantly affected by the inoculum. Previously published mycorrhizal responsiveness (growth response to mycorrhizal colonization) for *Aster ericoides* was

63.2%, meaning that growth increased significantly with colonization but could occur without it (Wilson and Hartnett 1998). Other closely related *Aster* species have also demonstrated significant biomass responses to mycorrhizae (Hartnett et al. 1994; Wilson and Hartnett 1998). Overall, the *Glomus* inoculum seemed to have a greater positive effect than the Mix inoculum both in terms of *Aster* growth and percent colonization. It might be fruitful to further investigate *Aster* response to *Glomus* colonization.

Across inoculum treatments, higher colonization in *Aster* led to higher biomass and higher root/shoot ratio. Researchers have speculated that root/shoot ratio in mycorrhizal plants may be related to soil nutrition (Hetrick 1991) or hormones produced or triggered in the host by AMF (Hetrick et al. 1988). Conventional thought indicates that AMF colonization lowers root/shoot ratios, as highly efficient mycorrhizal hyphae reduce the need for roots and free up resources for allocation to aboveground productivity. However, depending on the plant species in question, its root architecture, the fertility of the soil, and the AMF species involved, colonization by AMF has been found to increase, decrease or have no effect on root/shoot ratios (Hetrick 1991). For example, various studies of *Schizachyrium scoparium* have found increased root/shoot ratios for individuals grown in non-sterilized soils (Anderson and Roberts 1993), while other studies of *Schizachyrium* had found decreased or unchanged ratios (Seo et al. 1988; Meredith and Anderson 1992). In this study, *Aster* root allocation increased as AMF colonization increased, and had the greatest colonization and biomass in inoculum treatments that included the greatest quantity of *Glomus* species. Saunders et al. (1977) found that root/shoot ratios in onion varied when grown in association with different species of AMF. Further testing would help determine if root/shoot ratio in *Aster* was being influenced by an AMF species.

Overall, for these four species, at the tested rates, the commercial inocula tested did not appear to confer any significant benefit to the plants. Other studies have found the manufacturer's recommended rate for similar products insufficient to illicit a result (Rowe et al. 2007; Tarbell and Koske 2007): here, even twice the recommended rate was ineffective. Inoculum treatment did significantly affect plant productivity and percent AMF colonization in *Aster*, but in both cases none of the four treatments were significantly different from the control, raising questions about the ecological significance of the observed effect. If, however, the particular goal of a restoration was to boost the presence or competitive advantage of long-lived forbs, such as *Aster ericoides*, these inocula might be useful. It is possible that an inoculum treatment could be developed to specifically benefit conservative forbs. Such a product would, in time, increase the species diversity of prairie restorations.

Soil Responses

In contrast to the moderate effects of inoculum on grass growth and colonization, soil type had significant effects on the grass species studied. This is interesting given that both sites are listed by the USDA soil map as Chelsea fine sand, meaning there is no systematic difference between the sites in terms of soil texture, moisture holding capacity or nutrient availability. The contrasting effects of the two soil types used here likely come from land use history, which could affect resource availability and the soil microbial community.

Given the appearance of the dying *Monarda* seedlings, the tilled soil could have harbored more pathogens than the CRP soil. Alternatively, the greater AMF colonizing potential of the CRP soil may have conferred protection from soil-borne diseases, including agents associated with damping off (Gosling et al. 2006).

Although within CRP soils there was no relationship between percent colonization and plant performance, overall high colonization is associated with better performance. Grasses grown in CRP soil had better survivorship, productivity, and greater AMF colonization. In a study comparing warm- and cool-season graminoids, mycorrhizae were found to positively influence seed germination in cool season species, including *Elymus canadensis*, but not the warm season species (Hartnett et al. 1994). Survivorship in *Elymus* was not significantly affected by soil type in this study, but perhaps the lack of AMF or the presence of pathogenic fungi in the tilled soil negatively affected survivorship in the other plant species. Lower germination and survival rates in the tilled soil could also be the result of residual pesticides.

Tilling and monoculture practices utilized in conventional farming lead to declines in AMF diversity (Jansa et al. 2002; Jansa et al. 2003; Oehl et al. 2003; Gosling et al. 2006). Since many AMF species spread to new plant hosts by way of extraradical hyphae (Klironomos and Hart 2002), annual plowing and disking continually interrupts the hyphal network and may cause the AMF community to shift towards species that reproduce predominantly by spores instead of through hyphal contact (Gosling et al. 2006). Although certain species may survive tillage, it is unclear as to whether the AMF communities that remain in tilled soils, such as that used in this project, provide comparable benefits to the host plants (Johnson et al. 1992). Planting crops in monoculture also impacts the AMF community by severely reducing the number of possible host plant species. In a long term trial evaluating the effect of agricultural land use intensity on AMF communities, Oehl et al. (2003) found that soil from a mowed grassland had a higher diversity and density of AMF spores than soils from conventional moncultural sites. Even though the plant community supported by the CRP soil used in this study is not diverse (greater than 95% *Bromus inermis*), the fact that it has not been tilled in over 20 years has apparently allowed a more robust AMF community to develop, resulting in the increased colonization found in this study.

In contrast to the low levels of AMF colonization in the tilled soil, colonization of DSE was higher in the tilled ground. Distribution of DSE appears to be as widespread as AMF, both geographically and in terms of habitat (Mandyam and Jumpponen 2005). Therefore, it is not surprising to have found DSE in all of the plant species examined here. However, because so little is known about DSE, the observation that DSE were more abundant in the tilled soil raises more questions than it answers. It is unlikely that DSE are able to rapidly invade or survive the tillage of agricultural ground more successfully than AMF. Like AMF, sexual states for most DSE are unknown at this time (Jumpponen and Trappe 1998; Mandyam and Jumpponen 2005). How DSE reproduce or disseminate is also not understood (Jumpponen and Trappe 1998; Grunig et al. 2008). Some, but not all, species of DSE have been observed to produce conidia, but conidiogenesis appears to be rare and may require a prolonged exposure to cold conditions (Jumpponen and Trappe 1998). While it may be possible that reproduction occurs through conidia, this has not yet been verified. It has also been hypothesized that DSE may reproduce through microsclerotia (Grunig et al. 2008) or plant cells containing

DSE (Jumpponen and Trappe 1998). Regardless of how they spread, DSE have been implicated as being important in high stress environments such as sandy grasslands in Hungary (Kovacs and Szigetvari 2002; Mandyam and Jumpponen 2005). However AMF have been documented to be significant in similar habitats, so why would the DSE appear to thrive in favor of AMF in the tilled soils in this study? One known difference between DSE and AMF is that DSE are able to colonize a wider range of hosts, including many plant families considered to be non-mycorrhizal, such as Brassicaceae, and would therefore find hosts regardless of the crop (Mandyam and Jumpponen 2005). In spite of years of tillage and monocropping, the remaining DSE species would be able to colonize the all of host plants in this experiment, but it is possible that the most appropriate AMF species had been eliminated resulting in low AMF colonization in the tilled soils.

What does this mean for restoration?

From an AMF standpoint, it appears that site history appears to be an important consideration when selecting a prairie restoration site, suggesting that planting a prairie restoration into CRP ground is preferable to using a tilled agricultural site. Clearly, planting a restoration into the “blank slate” of tilled agricultural ground is appealing from a site preparation perspective. However, based on the data shown here, the benefits of a more robust AMF community may outweigh the inconvenience of site preparation. Another study conducted at the CRP site found that a single use of glyphosate (Roundup® Concentate) was sufficient to release native seedlings from *Bromus* competition and allow establishment (Bolwahn 2010). Several studies have demonstrated that AMF are not directly harmed by glyphosate, but can be indirectly affected as the host plant succumbs to the herbicide (Wan et al. 1998; Baumgartner et al.

2005; Savin et al. 2006). Thus, herbicide treatment followed by prairie plant seeding could result in higher AMF colonization for stabling native plants, even when planted into low-diversity CRP grassland sites.

For tilled sites with low densities of resident fungi or where a more diverse fungal community is desired the commercial inocula tested here do not look like a viable solution to returning AMF to the soil in prairie restorations. Even if producing inoculum from local remnants is labor intensive and disruptive, it appears to still be the most beneficial, especially since Klironomos (2003) found that native plants benefited the most from native AMF. However, even when local inoculum can be produced, the time before the restoration will be evenly colonized is unknown and most likely slow (Gosling et al. 2006). In spite of the fact that the importance of AMF in prairie communities is well known, an ideal method for introduction remains elusive.

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