EVALUATION OF ARBUSCULAR MYCORRHIZA POPULATIONS FOR ENHANCING SWITCHGRASS YIELD AND NUTRIENT UPTAKE

by

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EVALUATION OF ARBUSCULAR MYCORRHIZA POPULATIONS FOR ENHANCING SWITCHGRASS YIELD AND NUTRIENT UPTAKE

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University of Nebraska, 1996

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To enhance the economic viability of switchgrass (Panicum virgatum L.) as a forage or biomass crop, rapid stand establishment and high yields are needed using low inputs, particularly nitrogen (N) and phosphorus (P) fertilizer. Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with mycotrophic species, and can increase water and nutrient uptake by their host, and colonization by AMF may be essential for plant establishment and survival with mycorrhizal dependent plant species.

Arbuscular mycorrhizal fungal inoculum was collected from native and seeded switchgrass stands in Nebraska, Kansas, Iowa, Missouri, Virginia, and North Carolina. Four switchgrass cultivars were inoculated with AMF from each collection, and grown in sand cultures for 12 wk. Mycorrhiza inoculated plants produced 15-fold greater shoot and root yields, and recovered six-fold more N and 36-fold more P than non-mycorrhizal plants, indicating that switchgrass is highly mycorrhizal dependent. Plants inoculated with AMF from seeded switchgrass stands averaged 1.5-fold greater shoot and root yields than plants

inoculated with AMF from native prairies.

Switchgrass, big bluestem (Andropogon gerardii Vitman), sorghum (Sorghum bicolor L. Moench) and soybeans [Glycine max (L.) Merr.] were grown for 12 wk in sand cultures inoculated with three highly or three moderately effective AMF populations. Switchgrass and big bluestem responded similar to inoculation with the different AMF. However, sorghum and soybeans responses were different from switchgrass, suggesting that highly effective AMF associated with seeded switchgrass stands are not highly effective for these crops.

Switchgrass seedlings were inoculated with highly effective, moderately effective, or indigenous AMF populations, and grown in cone-tainers for 12-wk prior to transplanting in a Sharpsburg silty clay loam (fine montmorillonitic mesic typic Argiudoll) and an Ortello loam (coarse loamy mixed mesic udic Haplustoll) near Mead, NE. Switchgrass yield or N- and P-uptake were not significantly different between the introduced and the indigenous AMF at either site. This may have resulted from the inability of the introduced AMF to compete with the indigenous AMF for colonization of new switchgrass roots.

ACKNOWLEDGEMENTS

In his book, 'A Sand County Almanac', Aldo Leopold wrote,

> "I heard of a boy once who was brought up an atheist. He changed his mind when he saw that there were a hundred-odd species of warblers, each bedecked like to the rainbow, each performing yearly sundry thousands of miles of migration about which scientists wrote wisely but did not understand. No 'fortuitous concourse of elements' working blindly through any number of millions of years could account for why warblers are so beautiful. No mechanistic theory, even bolstered by mutations, has ever quite answered for the colors of the cerulean warbler, or the vespers fo the woodthrush, or the swansong, or - goose music. . . There are yet many boys to be born who, like Isaiah, 'may see, and know, and consider, and understand together, that the hand of the Lord hath done this."

It is by faith I believe God created the heavens and the earth (Hebrews 11:3), and my studies in agriculture, ecology, and the other sciences have further strengthened that faith, not questioned it. I strongly agree with the apostle Paul, when he wrote to the Romans, "Since the creation of the world God's invisible qualities - his eternal power and divine nature - have been clearly seen, being understood from what has been made (Romans 1:19)."

For me, the power of nature to proclaim God's beauty, wisdom, and power is beautifully described in the poetry of the Psalmist who wrote,

The heavens declare the glory of God;
the skies proclaim the work of his hands.
day after day they pour forth speech;
night after night they display knowledge.
There is no speech or language
where their voice is not heard.
Their voice goes out into all the earth,
their words to the end of the world.
(Psalm 19:1-4)

In this dissertation I want to acknowledge the Lord, Jesus Christ, who has given me the opportunities to pursue graduate studies, the talents and skills I applied and developed during my studies, and my faith, which has allowed me to see Him in all of creation. I also want to thank the Lord for my wife, Janet, for her love and support, and whom, outside of my faith and personal relationship with Jesus Christ, is His most precious gift to me (James 1:17).

INTRODUCTION

During the past 20 yr, switchgrass (*Panicum virgatum* L.) has been grown throughout the eastern and central US for pasture and hay production for livestock, soil conservation, and wildlife habitat. Recently, interest has also risen in growing switchgrass for biomass for energy production. The land area targeted for growing switchgrass as a biomass crop is land east of 100° Long. that is marginal or unsuitable for annual grain crop production because of high erosion potential or low soil fertility. The soils on these marginal croplands may also be low in arbuscular mycorrhizal fungal (AMF) propagules, or contain less efficient AMF species as a result of erosion and cultural practices.

To enhance the economic viability of switchgrass as a forage or biomass crop, rapid stand establishment and high yields are needed using low inputs, particularly nitrogen and phosphorus fertilizer. Research with other forage and grain crops suggests that adequate levels in the soil of effective AMF is essential for seedling establishment with obligate mycorrhizal species. In addition, AMF can increase water and nutrient uptake by their host plant. However, not all AMF isolates are equally effective. The objectives of this research were: 1) evaluate the level of dependence of switchgrass on AMF for nutrient uptake; 2) evaluate the effectiveness of different AMF for enhancing nutrient uptake and yield in switchgrass; 3) determine if highly effective AMF populations isolated from long-term seeded switchgrass

stands would also be highly effective with other forage and grain crops that could be grown in rotation with switchgrass; and 4) determine if switchgrass production and nutrient uptake in the field can be enhanced by inoculating seedlings with more effective AMF isolates.

This dissertation is arranged into five chapters, and follow the guidelines in the *Publications Handbook and Style Manual for the American Society of Agronomy*. Chapter 1 is a review of the literature on the role of AMF in plant and ecosystem processes, strategies for selecting effective AMF inocula, and strategies for field inoculation. Chapter 2 describes the studies used to evaluate the level of dependence of switchgrass on AMF, and the effectiveness of different AMF for enhancing nutrient uptake and yield in switchgrass. Chapter 3 describes studies used to determine if highly effective AMF populations isolated from long-term seeded switchgrass stands would also be highly effective with other forage and grain crops that could be grown in rotation with switchgrass.

Chapter 4 describes the results from two studies conducted to evaluate the effectiveness of different AMF populations collected from the rhizosphere of seeded and native switchgrass stands for corn and sorghum. The two experiments described in this chapter are flawed in that it was not possible to kill all of the indigenous AMF in the potting medium prior to inoculation with the AMF to be evaluated, thus confounding treatment effects. However, the

studies provide some useful information on the type of responses that may occur following introduction of non-indigenous AMF into a soil containing an indigenous AMF population. Chapter 5 describes studies used to determine if switchgrass production and nutrient uptake in the field can be enhanced by inoculating seedlings with more effective AMF isolates.

The appendix contains a table giving the composition and concentration of the nutrient solution used to fertilize the plants in the sand culture studies. In addition, the appendix contains a table showing the relative mycorrhizal dependence of switchgrass seedlings inoculated with the different AMF populations evaluated in the greenhouse experiments. The appendix also contains the skeleton ANOVA's and expected mean square tables used to determine appropriate F-tests used in analysis of the experiments described in chapters 2 through 5.

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CHAPTER 1

LITERATURE REVIEW

The Role of Arbuscular Mycorrhizal Fungi in Plant and Ecosystem Processes

Symbiotic arbuscular mycorrhizal fungi (AMF) are important at both the plant and ecosystem levels. At the plant level, AMF improve plant adaptation or tolerance to different environmental stresses, including low nutrient availability, water stress, rhizosphere pathogens and toxic elements in the environment. When these stresses are present in the environment, AMF have been shown to enhance nutrient uptake, particularly phosphorus (P), enhance water uptake, exclude or reduce the uptake of toxic elements, and modify the microbial composition of the rhizosphere, resulting in a reduction of pathogens or stimulation of beneficial microbial species (Perry and Amaranthus, 1990). At the ecosystem level, the coupling of plants with the soil and rhizosphere microbial processes is enhanced by AMF. Examples of ecosystem processes that are enhanced by AMF include nutrient cycling and soil development.

The benefits AMF provide in plant and ecosystem functioning are integrated across plant and ecosystem scales. For example, increased nitrogen (N) and P uptake by AMF at the plant level affects N and P cycling through the ecosystem. Thus, our understanding of and efforts to

manipulate the benefits AMF provide plants is hindered by the magnitude and complexity of processes AMF affect. Because of this limitation, the major benefits AMF provide plants, and attempts at manipulating AMF, will be reviewed primarily at the plant level. The role of AMF in ecosystem processes will also be addressed when relevent and sufficient information is available to provide insight into their function.

Enhanced Nutrient Uptake: Adaptation to Nutrient Stress

Nutrients supplied to the host plant by AMF are generally those that have low solubility and are relatively immobile in the soil. Although P has received the most attention, enhanced uptake of K^+, Mg^{2+}, Fe^{3+}, NH_4^+, and various micronutrients by mycorrhizal roots have also been reported (Sieverding, 1991; Barea et al., 1993). In addition, AMF will supply the plant with NO_3^-, even though this nutrient is very soluble in water and moves readily in soil solution (Johansen et al., 1993). In general, AMF enhance the uptake of a nutrient when plant demand exceeds the ability of the root to supply the limiting nutrient.

The mechanisms of nutrient uptake by AMF are not clearly understood. Prolific hyphal production by AMF is believed to result in greater exploitation of the soil volume surrounding the root. In addition, AMF mycelium are able to grow between soil particles and organic matter and

into voids that are much smaller in diameter than can be reached by roots. The secretion of enzymes by AMF and changes in soil pH around AMF hyphae may also increase nutrient availability, and rapid nutrient absorption by AMF could enable them to out-compete other soil microorganisms (Gianinazzi-Pearson and Smith, 1993).

The greater surface area per gram of root and the greater volume of soil exploited by the extra-matrical mycelium of AMF are believed to be the primary mechanism for enhanced nutrient uptake by mycorrhizal roots. Mycorrhiza absorb nutrients from outside the narrow nutrient depletion zone that surround the plant roots. As a result, AMF can enhance the absorption of nutrients which diffuse slowly through the soil, in addition to those that move readily by mass flow (Abbott and Robson, 1982). Mycorrhiza absorb P and other nutrients from the same nutrient pools and utilize the same forms of soil P that non-mycorrhizal plant roots utilize (Abbott and Robson, 1982). However, there is some evidence that AMF are able to absorb nutrients present in the soil at lower concentrations than plant roots (Killham, 1994).

The importance of AMF in nutrient acquisition has led to the concept of AMF as "biotic-fertilizers" (Menge, 1983), similar to symbiotic diazotrophic bacteria in the bacteria-legume symbiosis, which provide reduced N to the plant. However, this concept is misleading for two reasons. First,

AMF do not synthesize plant nutrients like diazotrophic bacteria synthesize NH_4^+ from N_2. The nutrients must already be present in the soil for AMF to absorb. Second, the bio-fertilizer concept misrepresents the role of AMF in natural, climax ecosystems. In climax ecosystems, an important ecological process is the establishment of efficient nutrient cycles and nutrient reserves. Concurrently, with climax plant species, which are usually perennials, the primary mechanisms of nutrient acquisition is through recycling of nutrients within existing biomass, with the absorption of nutrients from the soil being of secondary importance (Chapin, 1980). Mycorrhizae are a key component in maintaining the efficiency of these nutrient cycles by circumventing normal nutrient turnover processes. For example, hyphae from one mycorrhizal fungus may be growing in association with several different host plants at a site to form a common nutrient pool. Through hyphal links, nutrients can be transferred directly between plants (Newman, 1988; Frey and Schuepp, 1993). In addition, hyphae already present in senescing roots can absorb mineral nutrients before they escape into the soil solution or are absorbed by saprophytes, and transfer them to living roots, bypassing soil and microbial biomass pools (Ritz and Newman, 1985; Newman and Eason, 1989).

Another mechanism by which AMF facilitate the development and maintenance of efficient nutrient cycling is

through the formation of soil aggregates. Nutrient inputs from atmospheric deposition, weathering, biological fixation, and plant litter decomposition must become stabilized in soil organic matter and microbial biomass in order for nutrient reserves to accumulate (Miller and Jastrow, 1992). Organic residues are stabilized and protected in soil through the formation of stable soil aggregates. Both plant roots and soil microorganisms contribute to the formation and stabilization of both micro- (<0.25 mm diameter) and macroaggregates (>0.25 mm diameter). Physical entanglement by roots and hyphae of AMF is the major mechanism by which microaggregates are bound into macroaggregates.

Mycorrhiza contribute to the aggregation process through three closely related processes. First, the extraradical hyphae grow into the soil matrix and create a skeletal structure that holds the primary soil particles together through physical entanglement. The entangled particles are further bound together with organic debris to facilitate the formation of microaggregates. In the final phase, the microaggregates are physically enmeshed by extraradical hyphae and roots to create the macroaggregate structure (Miller and Jastrow, 1992). More conservative nutrient cycles result from the encapsulation of organic debris within soil aggregates. In addition, the formation of soil aggregates improves soil structure, resulting in

improved hydrological properties.

Exclusion of Toxic Elements: Adaptation to Edaphic Stress

Several studies have indicated that AMF have their greatest infectivity and effectiveness in soils with physical and chemical properties that are most similar to those they are indigenous to (Lambert et al., 1980; Brundrett, 1991). The observation that AMF possess specific adaptations to the soil in which they naturally occur lead Bethlenfalvay et al. (1989) to propose the term "edaphotype". They defined edaphotype as "intraspecific variants of these soil fungi that are of different edaphic origin and elicit distinct physiological responses from plants under uniform conditions." This phenomenon has proven beneficial because AMF isolates from adverse soils tend to stimulate greater host plant responses under the same adverse conditions, compared to AMF isolates from soils that lack the adverse conditions. Edaphic stresses AMF may ameliorate include very low (acid) or high (alkaline) soil pH, high salt concentrations (salinity) and heavy metals. However, exactly which soil stress the fungi are responding to is often not clear. If isolates from acid soils enhance nutrient uptake and stimulate greater plant growth in acid soils, the fungi may be responding to the high H^+ concentration or the greater Al^{3+}, Mn^{2+}, or Cu^{2+} availability resulting from low soil pH.

Because some AMF can tolerate high metal concentrations, they may also provide some protection from metal toxicity to their host plants. Koslowsky and Boerner (1989) collected AMF from a soil with a very high Al^{3+} concentration (253 ug/g) and from a soil with a low Al^{3+} concentration (<5 ug/g). In the greenhouse they grew switchgrass (Panicum virgatum L.) plants inoculated with either fungal population or a non-inoculated control, under three Al^{3+} concentrations (0.5, 2.0 or 5.0 mM Al^{3+}). The AMF inoculated plants had lower tissue Al^{3+} concentrations and absorbed less Al^{3+} than non-inoculated plants. This response was only partially compensated for in non-inoculated plants by increasing the soil P concentration 2.5-fold. The different AMF inoculi also affected switchgrass response to high Al^{3+} concentrations. Plants inoculated with AMF from the high Al^{3+} soil had significantly more tillers, shoot mass, and root length than plants inoculated with AMF from the low Al^{3+} soil. In addition, plants inoculated with AMF from the high Al^{3+} soil had significantly lower tissue Al^{3+} than plants inoculated with AMF from the low Al^{3+} soil.

The exact mechanism by which AMF improve plant tolerance to high metal concentrations is unknown. One hypothesis is that during nutrient absorption fungal hyphae selectively exclude metals, or excrete those that are absorbed by mass flow. As part of the exclusion mechanism,