A P P L I C A T I O N

The Effects of Mycorrhizal Inoculant and Micronutrient Seed Coating
on Early Plant Establishment on a Tallgrass Prairie Reconstruction

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Sponsoring agency or department (if applicable):

State (Agency)__________ County (Name & no.) __________

City __________ College/University __________

***Note*:**
If private citizens or groups are proposing a roadside enhancement project, a letter of support from the agency maintaining the right-of-way must be attached.

2. Purpose of application: (Choose most appropriate, or if multipurpose, please rank—with 1 being the highest priority.)

Roadside inventory	_____
Gateway landscaping/Roadside enhancement	_____
Research, demonstration, and education	___X__
Equipment	_____
Other (list below)	_____

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3. Maintenance of roadside, gateway, or other project site (if applicable):

Person Responsible: __________ Phone Number:__________

Maintenance of equipment(if applicable):

Person Responsible: __________ Phone Number:__________

The Effects of Mycorrhizal Inoculant and Micronutrient Seed Coating on Early Plant Establishment on a Tallgrass Prairie Reconstruction

Proposed Research

This is a two-year research project to determine the effect of mycorrhizal fungal inoculants and micronutrients on early establishment, species composition, weed competition, and viability of a native tallgrass prairie reconstruction. The results of this study will be helpful in determining if using commercially available mycorrhizal fungal inoculants and/or micronutrient applications should be used in prairie reconstructions.

This is a two-year project. The proposal is for the second year of the project.

Introduction

Prairie reconstructions are often done in areas that have experienced high levels of soil disturbance such as cropland, right-of-ways, and turf grass. Soils disturbed by tillage, grading, compaction, chemical application, and other human activities are often deficient in arbuscular mycorrhizal (AM) fungi that are needed for optimal establishment and growth of plants. This is especially important in soils with low nutrient concentrations, particularly phosphorous (Amaranthus and Steinfeld 2003, Entry et al. 2002). Also, such soils may lack adequate quantities of the micronutrients needed for efficient plant growth and development. Recently the cost of mycorrhizal inoculants, which use fungal spores to add AM fungi back into the soil community, has been reduced making its use for prairie reconstructions more feasible. Planting and restoring prairies can be expensive and it may prove to be more cost effective to use inoculum and/or micronutrients to maximize planting success.

Arbuscular fungi and plants form a symbiotic relationship. It has been estimated that mycorrhizae will form symbiotic associations with 70-90% of all plant species making it an essential component of prairie ecosystems (Brooks et al. 2006). The fibrous root-like hyphae of AM fungi attach themselves to the plant roots and form arbuscules within the cortical cells which allows the fungi to obtain carbon from the plant (Bever et al. 2001). In return, the fungi benefit individual plants by transferring water and nutrients thus aiding the plant in a multitude of ways such as increasing the surface area of the root system, decreasing susceptibility to drought, increasing access to nutrients, increasing nitrogen fixation in legumes, improving soil structure, increasing disease resistance, and decreasing the likelihood of damage by nematodes (Brooks et al. 2006). It has been estimated that the nutrient uptake by the hyphae of AM fungi can supply up to 80% of the phosphorous and 25% of the nitrogen required by an individual plant (Wilson et al. 2001).

Micronutrients are needed in trace amounts for a plant's metabolic processes to run efficiently. These nutrients can act either as synthetic enzymes or as a cofactor to activate an enzyme, or they can be used to aid the movement of sugars through plant tissue. Cobalt is an example of a micronutrient that is used in the root nodules of legumes to permit nitrogen fixation (Carpenter, Tarr, and Rice 1980). Adding these micronutrients to a prairie seeding may increase the metabolic efficiency of the plants, thus increasing the robustness of the prairie during the early stages of reconstruction.

Most of the studies demonstrating the effect of mycorrhizae on individual plant species have been done in the greenhouse. A study performed at Konza Prairie in northeastern Kansas by Hartnett and Gail (1999) found that reducing the amount of mycorrhizal colonization by applying fungicide greatly reduced the dominance of the C_4 grasses. It is clear that the presence of AM fungi and micronutrient availability have widespread effects on the plant community of a prairie.

Objectives

This 2-year project will investigate the following questions:

1. Does inoculating native prairie seeds lead to a higher rate of colonization by AM fungi?

2. Does AM fungi colonization increase seedling emergence and establishment?
3. Does AM fungi colonization increase root mass?
4. Does AM fungi colonization increase above ground plant growth?
5. Does AM fungi colonization affect soil nutrient levels?
6. Does availability of micronutrients increase early growth and establishment?
7. Does availability of micronutrients increase colonization rates of AM fungi?
8. Does the presence of micronutrients and/or AM fungi affect plant mortality?
9. Does the presences of micronutrients and/or AM fungi increase species diversity and richness?
10. Does the presence of micronutrients and/or AM fungi reduce weed competition during establishment?

Methods

The research site is a 2-acre portion of an 8 acre field on the western boundary of the resource area. The soil type is a Spillville-Coland soil complex. The site was in soybean production in 2008, and has been sprayed with glyphosate for weed suppression. This experiment will utilize a random block design with two blocks. Within each block there will be four treatments that will be repeated three times each for a total of 12 (17.75 m x 17.5 m) plots in each block and 24 plots total. Soil samples will be taken from each plot and analyzed for phosphorous, nitrogen, potassium, trace elements, and organic carbon content. The four treatments are 1) control which will receive no treatments, 2) mycorrhizal inoculant treatment, 3) micronutrient application treatment, and 4) mycorrhizal inoculant and micronutrients combination treatment. The prairie seed will be Iowa source-identified seed purchased from local growers and will consist a mixture that includes 6 warm-season grass species, 2 cool-season grass species, 7 legume species, and 21 forb species. Seeding will be done with a Truax no-till seed drill, and the research area will be mowed during the first growing season at a height of 4”-6” to reduce weed competition.

Sampling will be conducted in June 2009, September 2009, and June 2010. Data will be collected from fifteen ¼ m^2 quadrats placed randomly in each block. Species presence and coverage will be determined. Above ground and below ground biomass samples will be taken and samples of roots from all plots will be analyzed for percentage colonization using the grid-intercept method. Simpson’s Diversity Index will be used to compare variation in plant diversity between the control and the treatments. Soil samples will be taken after the final vegetative sampling and compared to the pre-planting samples. Individual plant mortality data will be determined by placing permanent rings around individual plants and observing their persistence from year one to year two. The data will be analyzed using a 2-way ANOVA and Tukey’s protected test for pairwise comparisons to compare means among the various treatments.

Revised Timeline

Initial soil sampling	May 2009
Plot seeding	May 17th and 18th, 2009
Plot establishment mowing	June, July, August 2009
First vegetation sampling	September 2009
Second vegetation sampling	June 2010
Third vegetation sampling and root sampling	August 2010
Second soil sampling	August 2010
Final LRTF report	December 2010

Project Progress

The design of the research site at the Cedar River Natural Resource Area in southeastern Black Hawk County is completed; the plots were measured and marked in the fall of 2008. The plots are 17.5 m x 17.5 m with a 3 m buffer between plots. The corners of each plot are marked with a 30 cm x 30 cm - ¾" treated plywood square fastened with a rebar stake through the middle. A paint pen was used to write the plot number and treatment type on each plot marker. GPS coordinates will be recorded for each plot using a Trimble receiver and plotted using ArcPad software.

The site was in soybeans last growing season and was sprayed twice with glyphosate for weed suppression. Currently the site is mostly devoid of any vegetation with the exception of a few invasive annuals and biennials. Garlic mustard is the predominate species, but it will not persist in the planting as the native species become established. Establishment mowing will help reduce the competitive effects of invasive species on native species.

Soil samples were taken on May 2009 and sent to the Iowa State University Soil Testing Lab for analysis. The soil sample to be tested was obtained by taking 20 random soil cores that were 15 cm deep and 4 cm in diameter. The 20 cores were broken up and thoroughly mixed before sending to the lab for testing for nitrogen, phosphorous, potassium, and micronutrient levels. The purpose of the soil sample was to provide a general characterization of the soil nutients of the site as they can affect the benefits provided by the mycorrhizae.

The mycorrhizal inoculant (Myco Apply Endo in granular form) was purchased from Mycorrhizal Applications Inc. This inoculant contains *Glomus intraradices*, *G. mosseae*, *G. aggregatum*, and *G. etunicatum* and is certified to contain 60,000 propagules per pound. The micronutrient (Nutriplant SD 0-0-0) to treat the seeds was donated by the distributor. The company claims that the micronutrient powder containing Ca, Mg, S, Co, Cu, Fe, Mn, Mo, and Zn will enhance seedling emergence and growth.

Seed was purchased from three different local growers. The majority of the seed purchased for the seeding mixture was Iowa source-identified (yellow tag). Yellow tag seed was not available for some species and in those cases Iowa origin seed was purchased. Species composition and details of the seed mix are included in Table 1. Prior to planting, the seed for each plot was weighed by individual species and mixed. The mycorrhizal inoculant and micronutrient powder was thoroughly mixed with the seed for those treatments. The mycorrhizal inoculant was applied at a rate of 20 lbs. per acre as recommended by the manufacturer for reconstructions planted with a no-till drill. The powdered micronutrient seed coating was added at a rate of 3.5 oz. per 100 lbs. of seed as per the label rate for the planting of grain crops.

Table 1. Seed mix for 2.5 acre mycorrhizae/micronutrient experiment

Grasses		**Phenology**	**seeds/sq.ft**	**Cost(2007)**
Big Bluestem	Andropogon gerardii	Warm-season grass	8	174.24
Side-oats Grama	Bouteloua curtipendula	Warm-season grass	4	100.72
Prairie Brome	Bromus kalmii	Cool-season grass	2	54.45
Canada Wildrye	Elymus canadensis	Cool-season grass	2	105.39
Switchgrass	Panicum virgatum	Warm-season grass	8	163.35
Little Bluestem	Schizachyrium scoparius	Warm-season grass	5	106.76
Indian Grass	Sorghastrum nutans	Warm-season grass	5	94.70
Tall Dropseed	Sporobolus asper	Warm-season grass	3	32.67
		TOTAL(grass)	**37**	**$832.27**
Forbs				
Leadplant	Amorpha canescens	Legume	1	73.07

Thimbleweed	Anemone cylindrica	Forb	0.25	99.09
Prairie Sage	Artemisia ludoviciana	Forb	2	17.42
Butterfly Milkweed	Asclepias tuberosa	Forb	0.5	243.81
New England Aster	Aster novae-angliae	Forb	1	24.20
Milk Vetch	Astragalus canadensis	Legume	3	70.02
White Wild Indigo	Baptisia leucantha	Legume	0.25	128.12
Partridge Pea	Cassia fasiculata	Legume	2	242.00
Prairie Coreopsis	Coreopsis palmata	Forb	0.25	49.50
Purple Prairie Clover	Dalea purpurea	Legume	3	51.72
Showy Tick Trefoil	Desmodium canadense	Legume	1	99.00
Pale Purple Coneflower	Echinacea pallida	Forb	1	102.74
Rattlesnake Master	Erynigium yuccifolium	Forb	0.5	58.08
Ox-eye Sunflower	Heliopsis helianthoides	Forb	1	51.86
False Boneset	Kuhnia eupatoriodes	Forb	1	34.03
Round-Headed Bush Clover	Lespedeza capitata	Legume	0.5	72.60
Rough Blazingstar	Liatris aspera	Forb	1	140.52
Wild Bergamot	Monarda fistulosa	Forb	2	29.04
Wild Quinine	Parthenium integrifolium	Forb	0.5	93.34
Foxglove Beardtongue	Penstemon digitalis	Forb	1.4	5.86
Prairie Phlox	Phlox pilosa	Forb	0.25	85.97
Common Mt. Mint	Pycnanthemum virginianum	Forb	3	44.55
Yellow Coneflower	Ratibida pinnata	Forb	3	43.56
Black-eyed Susan	Rudbeckia hirta	Forb	2	7.10
Compass Plant	Silphium laciniatum	Forb	0.1	165.00
Stiff Goldenrod	Solidago rigida	Forb	3	63.75
Prairie Spiderwort	Tradescantia bracteata	Forb	0.5	136.13
Golden Alexanders	Zizia aurea	Forb	2	118.80
		TOTAL(forb)	**37**	**$2,350.88**
		TOTAL (grass & forb)		**$3,183.15**

Originally the seeding time was scheduled for late fall 2008. A $750 grant was received from the College of Natural Sciences to defray some of the pre-seeding costs. However, the seed could not be ordered until the LRTF grant was approved after Nov. 1, 2008. By the time the seed vendors filled the orders and delivered the seed, the ground was covered with snow. The seeding time was then switched from a fall planting to a spring planting and the research plots were seeded May 17th and 18th, 2009. All plots were planted using a 4-foot Truax no-till drill. Prior to seeding the drill was recalibrated to ensure equal distribution of seed throughout each individual plot. Crushed clay chips were added to the seed mixture in the plots that did not contain the mycorrhizal inoculant. The purpose of this was to equalize the flow rates of seed through the drill for all treatments as the granular mycorrhizal inoculant increased the rate of seed flow for that treatment.

Establishment mowing will take place as needed throughout the first growing season and during the second growing season if necessary. Mowing at a height of 4”- 6” will help to reduce competition of exotic and invasive species and will increase light availability to native seedlings. If any perennial exotic or invasive species become established in the plots they will be either pulled or sprayed.

Due to the spring seeding date, sampling will occur during September 2009, June 2010, and August 2010. Sampling will consist of seedling counts in 15 randomly placed ¼ m^2 quadrats within each plot. Of the 15 quadrats, 5 will be randomly selected for aboveground biomass sampling. After clipping the biomass plots, the vegetation will be sorted into native grasses, native forbs, and weeds. The biomass will then be dried, weighed, and recorded for data analysis. During the final sampling root sample plugs will be taken for all plots and fungal root colonization will be determined using the gridline-intercept method in order to assess the effectiveness of the mycorrhizal inoculant and to determine the amount of natural recolonization in non-inoculated plots. Simpson's Diversity Index will be used to determine if any of the treatments had an effect on species richness and diversity. Data will be analyzed using a 2-way ANOVA and Tukey's protected test for pairwise comparison of means among treatments.

Education and Outreach

The purpose of this research project is to further the knowledge and understanding of native prairie ecosystems. The information gained by this study will be useful to agencies and individuals, public or private, involved with prairie reconstruction, restoration, or management. A preliminary report and research design was presented at the 2009 annual Iowa Academy of Science meeting. Upon completion of the project, a summary report will be submitted to the LRTF committee and the results will be presented at the 22nd North American Prairie Conference, Iowa Prairie Conference, Iowa Academy of Science, and the Iowa Roadside Conference. The Tallgrass Prairie Center will also be able to use this research site for demonstrations during workshops, conferences, and field trips for the general public. The site will be maintained and managed by the Tallgrass Prairie Center and the Black Hawk County Conservation Board.

Budget

	SOURCE OF FUNDS	
	LRTF	TPC-UNI
Personnel		
Research Administrator (5% time)		
Salary	$ 2,700	
Fringe Benefits	$ 1,004	
Graduate student stipend	$ 8,392	
Graduate tuition		$ 7,596
Student salaries	$ 6,808	
Sub-Total	$18,904	
Supplies and Services		
General	$ 500	
Travel	$ 900	
Sub-Total	$ 1,400	
Total Direct Cost	$20,304	
Indirect Costs (8%)	$ 1,624	
Total LRTF Request	**$21,928**	
Match	$ 7,596	
Total Project Cost	$29,524	

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Vita

Vita provided with Year 1 Proposal

The Effects of Mycorrhizal Inoculant and Micronutrient Seed Coating on Early Plant Establishment on a Tallgrass Prairie Reconstruction

Chris Barber

Living Roadway Trust Fund Interim Report

Project Progress Since Last Report

Seed for this experiment was weighed out by percentage pure live seed (PLS) and according to the number of seeds per square foot in the seed mixture for each individual plot. This ensured that the amount of seed of each species remained constant throughout all plots. The mycorrhizalinoculant and micronutrient seed coating was mixed with the seed at rates recommended by the manufacturers for restoration or pasture applications. Inert crushed clay chips of roughly the same size as the granules of the mycorrhizalinoculant was added to the control and micronutrient seeds to homogenize the rate of flow for all treatments. Without the clay chips these treatments would form clumps of seed reducing the uniformity of seed dispersal.

Planting was performed in mid-May 2009 using a 4’ Truax no-till grass seeding drill. The drill was fitted with a custom tube attachment in the seed box for planting small quantities of seed ensuring that all seed gets planted in each plot. Special care was taken during planting to not drive across plots that had already been planted in order to reduce the amount of disturbance and cross contamination of treatments. The order of planting was control, micronutrient treatment, combination treatment, and finally the mycorrhizae treatment. This order eliminated contamination of mycorrhizalinoculant in the drill. The drill was thoroughly cleaned between the combination treatment and the mycorrhizae treatment to keep any residual micronutrient powder left in the drill tubes from contaminating the mycorrhizae treatments. The buffer strips between plots was planted in a cool-season pasture mix.

The 2009 growing season proved to be an excellent year for prairie establishment in this area. Periodic rains and cool summer temperatures benefited the establishing native seedlings. Weed pressure at the Cedar River Natural Resource Area was fairly minimal in part to its sandy alluvial soils. Because of this establishment mowing was only required twice during the growing season, once in mid-June and once in late July. Plot buffers were mowed periodically with a turf mower to prevent the pasture mix from going to seed.

Initial vegetation sampling was conducted the 27th and 28th of July. Each plot was sampled by counting the number of seedlings for native and weed species found within a 0.1 m^2 quadrat. Fifteen random sites were sampled in each plot and a sampling buffer of 1 m was excluded from the perimeter to eliminate edge effects in the sample site. For the purpose of this experiment, seedlings that were not in the seed mixture were considered weeds regardless if they were native species or not.

Five of the quadrats were randomly selected to be analyzed for biomass production. After the number of seedlings was recorded the vegetation was clipped 1" above the surface and separated into native grasses, native forbs, and weeds. The vegetation was placed in paper bags and dried in a drying oven until a constant mass was achieved. Each sample was then weighed and recorded.

During a mid-August mowing of the plot buffers it was noticed that certain forb species were beginning to flower in some treatments more so than others. To quantify this each plot was divided into four equal sections and a transect was walked down the middle of each section effectively covering the entire plot. While walking each transect every flowering native was counted and the species recorded. A seedling was considered to be flowering if any portion of the petals was visible.

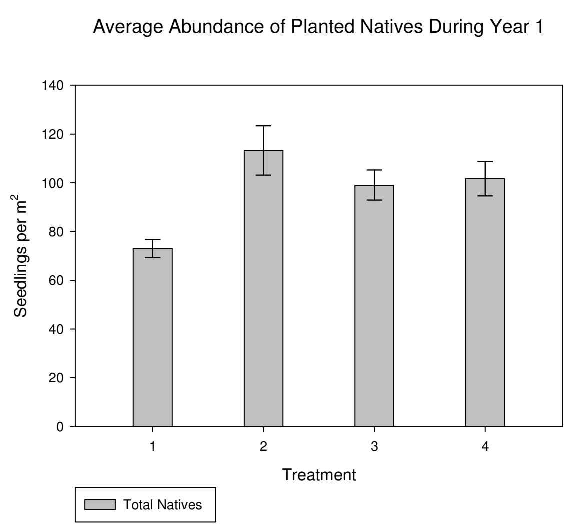
Initial Results

Data was analyzed using ANOVA and Tukey's protected test for pairwise comparison to compare means among treatments. No statistical differences were found between blocks so the data was combined. It was found that the mycorrhizalinoculant increased the total number of native seedlings by 35% while the micronutrient and combinations increased native abundance by approximately 25% each. When broken down into warm-season grasses, cool-season grasses, and forbs the largest differences were in the warm-season grasses. Mycorrhizal treatments had 32% more warm-season grass seedlings than the control while the micronutrient and combination treatments had 26% and 29% respectively. There was no statistical difference in the number of cool-season grasses present. Forb abundance was broken down into legumes and non-legumes and no statistical differences were found between the two groups. Mycorrhizal treatments had approximately 43% more forbs than the control while the micronutrient and combination treatments were not significantly different although an increasing trend was present.

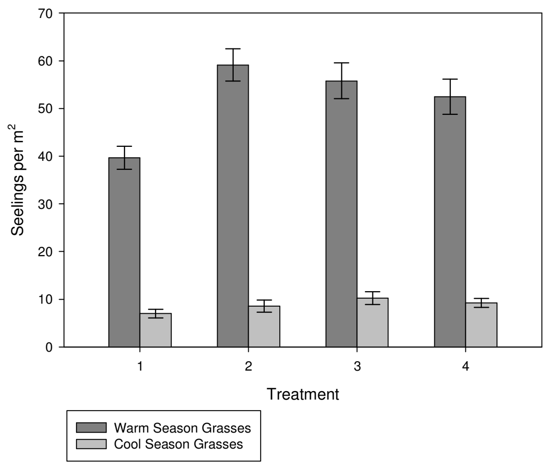
Analysis of the flowering time data revealed that all treatments experienced an increase in the number of flowering forbs present over the control. This biggest difference by species was in partridge pea which was the most abundant flowering plant. The mycorrhizal treatments had 39% more flowering partridge pea than the control and the micronutrient and combination treatments were at 33% and 36% respectively. There was a similar trend in the number of flowering black eyed Susan seedlings although it was not statistically significant.

There were no statistical differences in either total biomass or biomass of grasses, forbs, or weeds among treatments although there was a trend that mycorrhizaltreatments had increase in grass biomass and reduced weed biomass. The lack of statistical significance may be due to the high degree of variance as a result of the early stage in establishment and a small sample size which will be increased in future samplings.

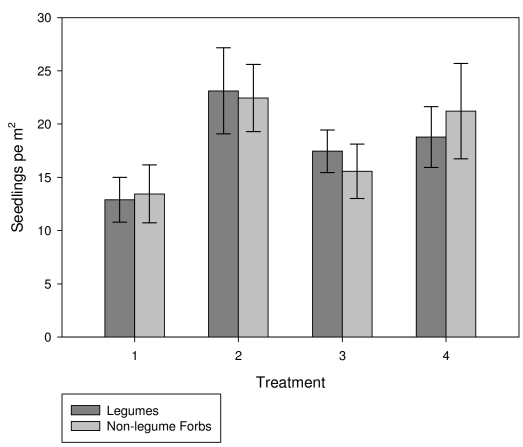
In all graphs: Treatment 1 = Control
Treatment 2 = Mycorrhizal inoculant
Treatment 3 = Micronutrients
Treatment 4 = Mycorrhizal inoculant and micronutrients



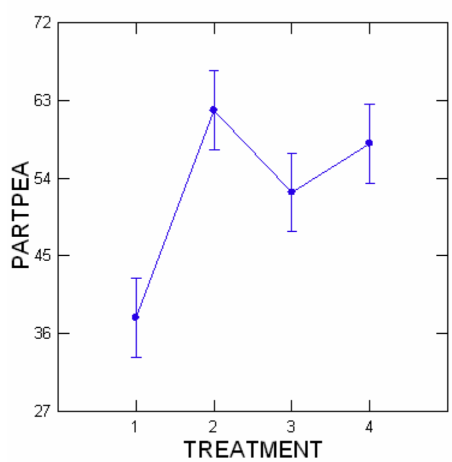
Average Abundance of Grass Seedlings During Year 1



Average Abundance of Forb Seedlings During Year 1



Least Squares Means



Partridge pea flowering in mid-August 2009

Continued Research

Vegetative sampling will be collected again in June 2010. This sampling will be conducted in the same fashion as the previous sampling but the number of biomass sampling will be increased from five to ten. Also, during this sampling period soil cores will be taken and sorted for root material which will be stained and analyzed for the percentage of root colonization by mycorrhizal fungi using the gridline-intercept method.

In addition, a greenhouse experiment will be conducted during the winter of 2010. The purpose of this experiment is to develop a growth curve for big bluestem with and without mycorrhizal colonization under varying concentrations of soil phosphorus. The effectiveness of mycorrhizae to provide a benefit to the plant is greatly impacted by amount of plant available phosphorus. At high concentrations the plant can obtain all the phosphorus that it needs on its own but if mycorrhizae are present they are still giving up the products of photosynthesis. At the end of the experiment the soil will be analyzed for phosphorus content in the same manner a field soil test would be performed. This should yield a practical guide as to at what level of soil phosphorus it would be beneficial to a prairie reconstruction to add mycorrhizalinoculant.