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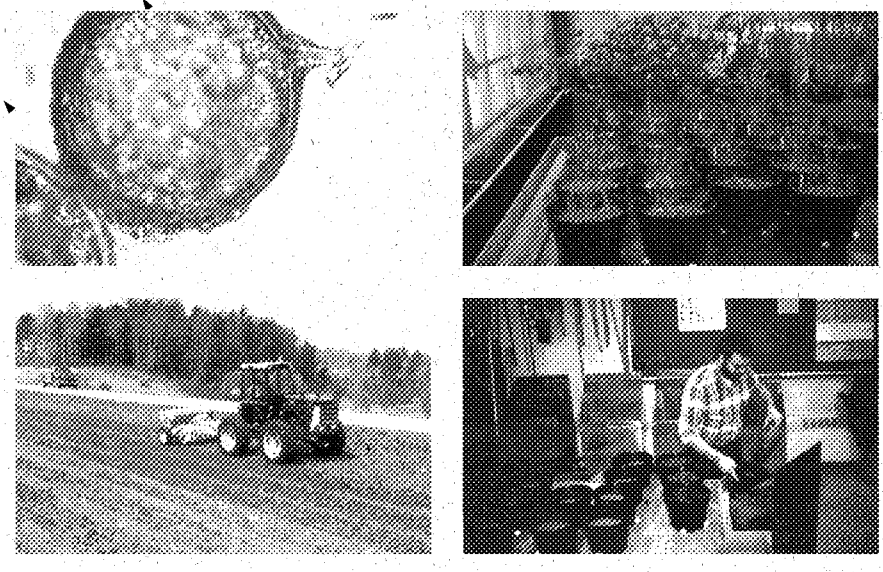

Culture and Re-Introduction of Vesicular Arbuscular Mycorrhizae in a Prairie Restoration

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16. Abstract (Limit: 200 words)

This project sought to examine whether it was possible to re-introduce naturally occurring soil fungi called vesicular arbuscular (VA) mycorrhizae back into disturbed soils as part of a normal seeding operation for restoring a prairie. The project consisted of three phases; 1) a survey to determine the types of VA mycorrhizae occurring in different soils and vegetation types (i.e. general roadside, prairie remnant, prairie planting); 2) collect from the wild and then grow prairie VA mycorrhizae species in a greenhouse at the University of Minnesota; 3) install the VA mycorrhizae along with seed from a conventional native seed drill and monitor whether the cultured VA mycorrhizae formed mycotrophic associations with prairie species in the field. This project was a preliminary feasibility study and was not intended to answer long term questions regarding the possible benefits VA mycorrhizae may confer to roadside plantings or other prairie restorations.

All phases of the project were successful. The survey indicated that differences in VA mycorrhizae composition exist between different sites. It is possible to produce VA mycorrhizae in a greenhouse by culturing on living plant hosts. The VA mycorrhizae produced was successfully installed along with seed into field plots and did form mycotrophic associations with newly planted prairie species. Preliminary results obtained from this project indicate that further investigation is warranted.

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CULTURE AND RE-INTRODUCTION OF VESICULAR ARBUSCULAR MYCORRHIZAE IN A PRAIRIE RESTORATION

FINAL REPORT

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EXECUTIVE SUMMARY

The Minnesota Department of Transportation (Mn/DOT) is expanding the use of native species in replanting public lands under it's jurisdiction. Native grasses and forbs (flowers) are currently being used in a number of places by Mn/DOT; including along roadsides and rest areas following new construction, for re-habilitating weed infested areas, and in sites to be restored as part of wetland mitigation. The objective in most cases is to re-establish a vegetative cover that is a functioning natural plant community, which in turn may reduce roadside vegetation maintenance significantly. The State has put a considerable amount of effort into developing commercial seed sources for native grass and forb species in Minnesota and in the development of management practices for lands where native species have been re-established. One thing that has been neglected in efforts to restore areas to native vegetation is below ground in the soil where it has been well documented that plants interact with various soil microbes and fungi. In fact, the majority of Mn/DOT projects have soils that are disturbed, or manufactured, and are likely devoid of beneficial microbes and fungi that are specifically associated with the native species being used to re-plant these areas.

The objective of this project was to perform a brief survey of native prairies looking for soil fungi called vesicular arbuscular (VA) mycorrhizae that are known to be associated with native grasses such as big bluestem and little bluestem which are commonly planted by Mn/DOT and compare them with disturbed sites to see if the same species occur in both areas. We also wished to determine whether small quantities of VA mycorrhizae inoculum could be increased by laboratory culture to amounts sufficient to use in a restoration. A prerequisite was that inoculum produced had to be able to be installed with seed using conventional seeding equipment. Finally, we wished to determine whether an area that was re-seeded with native prairie species and inoculated with VA mycorrhizae would exhibit colonization of plant roots with the associated VA mycorrhizae fungus.

We found that native prairie soils contain a high diversity of VA mycorrhizae species that are associated with prairie plants and that disturbed soils also contained VA mycorrhizae. However, even though disturbed and undisturbed areas both contain VA mycorrhizae, there appeared to be a difference in the diversity and types of VA mycorrhizae which occur in disturbed versus undisturbed soils. In the

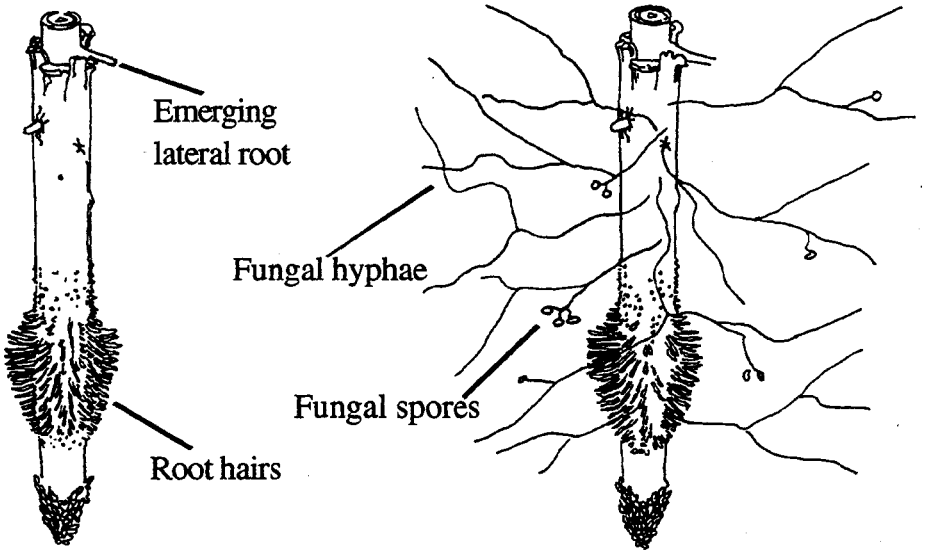
laboratory we were able to cultivate VA mycorrhizae using native grass hosts and produce a sufficient amount of material to inoculate approximately 10 acres of a prairie restoration. In the field study we found that inoculum was easily installed with seed using a Truax native grass drill and subsequently native grasses were colonized by VA mycorrhizae. Being that this project was a one year field study, we were not able to determine the long-term outcome of whether or not re-introduction of VA mycorrhizae associated with prairie species actually confers benefits in species diversity, adaptability and survival. We do however, have experimental areas that can be monitored and sampled in the future in order to make some conclusions on longer term effects or benefits.

Results of this project demonstrate that it is possible to culture naturally occuring VA mycorrhizae associated with prairie species in a laboratory setting and successfully re-introduce it back into a restoration. It is anticipated that the preliminary results derived from this study may have potential application for the restoration of natural landscapes by both government and private organizations.

CHAPTER 1. INTRODUCTION

Nearly all species of higher plants form a symbiotic association with mycorrhizal fungi which can benefit both plant and fungus. In this association the fungi germinate in the presence of a plant root and produce hyphae that penetrate the intercellular cortical region of the root. Referred to as mycotrophy, this association facilitates increased soil exploration for nutrients and water by plants (Figure 1) and may prevent deleterious fungal and bacterial establishment on host plant roots. The result of the association allows survival of both plants and fungi under conditions where both may perish separately.

Figure 1. Comparison Between a Mycorrhizal and Non-mycorrhizal Root



Non-mycorrhizal Root **Mycorrhizal Root**

*Adapted from Bonfante - Fasolo, 1984.

In prairies, mycorrhizal fungi may play an important role in ecosystem functioning [1, 2, 3]. It has been documented that most prairie grasses and forbs are extensively colonized by vesicular arbuscular mycorrhizae (VA mycorrhizae) [4, 5, 2]. In addition, laboratory research has shown that prairie plants benefit from symbiotic associations with VA mycorrhizae [5, 6].

The Minnesota Department of Transportation (Mn/DOT) replants disturbed areas along roadsides, at rest areas, and at sites being restored as part of wetland or other types of mitigation. Mitigation sites are frequently transfered to the Minnesota Department of Natural Resources (DNR) for future designation as wildlife management areas (WMAs). Most Mn/DOT restoration projects now involve the re-establishment of native species, especially prairie plants which can be managed using prescribed burning, because they are uniquely adapted to survive fires [7, 8]. It is anticipated that the management of of prairie species along roadsides using fire will allow Mn/DOT and county highway departments to eventually phase out mowing and herbicides as their primary management tools in many areas of the state [9]. It is hoped that the inclusion of VA mycorrhizae as a component of prairie restorations will improve the success of re-establishing more complete native prairie communities and ecosystems in Minnesota.

This project was divided into three successive phases; 1) a VA mycorrhizae field survey and collection, 2) VA mycorrhizae culture in the laboratory, and 3) re-introduction of VA mycorrhizae into field plots. Implementation of each phase was contingent on the success of the previous phase. Our primary objectives in this project were to:

1. Perform a survey of prairie mycorrhizae in southwestern and central Minnesota to identify possible differences in species composition, diversity and/or distribution between different sites,
2. Develop methods for culturing prairie mycorrhizae for re-introduction into roadside and other disturbed soils,
3. Determine the feasibility of mycorrhizal introduction to soils using conventional seeding methods,
4. Determine whether or not re-introduced mycorrhizae formed mycotrophic associations with prairie species seeded into field plots.

CHAPTER 2. PROJECT PHASE 1: MYCORRHIZAL SPORE SURVEY

2.1 Phase 1 Overview

The survey phase of this project addresses the need to identify the types of VA mycorrhizae present in native prairies (both along roadsides and off-road) because natural distributions of VA mycorrhizae species appear to be influenced by edaphic factors [10] and plant community composition [11]. We also wished to know if the VA mycorrhizae species found in native prairies were different than those found in roadsides planted with introduced grasses such as smooth brome (*Bromus inermis*) or in cultivated farm fields.

2.2 Materials and Methods.

2.2.1 Survey

In November of 1991, straight-line transects were established in various prairie and non-prairie sites, including restored and remnant prairies. Five subplots were sampled, separated by 50m along the transect. A composite soil sample was removed from each plot using a soil corer (2 cm diameter x 12 cm; 3 cores per plot). The soil samples were placed in a labeled plastic bag for transport in a styrofoam cooler to the laboratory.

2.2.2 Spore Isolation

After complete dry-down in a forced air oven set at room temperature, a 25 gram homogenized sample was obtained. Spores were isolated using a method adapted from McKenney et al [12], (see Appendix A). Remaining core soil samples were placed in labeled boxes and kept at room temperature for long-term storage. Subsamples of the soil are available upon request. The fungi were identified to the species level [13, 14]. Voucher slides [15] are stored at the University of Minnesota Plant Biology Department (Room 762) and are available for examination.

2.2.3 Statistical analysis

Analysis of variance was conducted using Statview SE+Graphics, v. 1.03 (Abacus Concepts, Inc., 1988). Spore population data were log(1+x) transformed [16] prior to analysis. Mean separation was determined using the least significant difference (LSD) criteria.

2.3 Results & Discussion

A number of different sites located around the state were surveyed (Table 1). Mycorrhizal spores were recovered from all survey sites (Table 2). The greatest number of spores per gram of dry soil were obtained from the Fuller Lake Rest Area (FLRA) and the railroad right-of-way near the Mn/DOT Materials and Research Lab (MRL-RR) with 194 and 232 spores, respectively. No significant difference exists in total spore number between these two sites but there is a difference in species composition. The greatest contrast was between the small yellow and brown type spores. *Glomus geosporum* was much more abundant at the MRL-RR site.

Table 1. Survey Sites

Name of Site	Type	County
November 1991		
• Fuller Lake Rest Area	Prairie planting 1972	Stearns
• Crosstown Prairie	A. Disturbed remnant prairie B. Backslope region between remnant prairie and Highway 55	Hennepin
• University of Minnesota Horticulture Research Prairie	Native grass planting	Ramsey
• Feder Prairie	Remnant prairie	Blue Earth
• Mn/DOT Materials & Research Laboratory	A. Prairie planting 1991 B. Adjacent abandoned railroad right-of-way	Ramsey
May 1992		
• Lake Shetek wetland/prairie restoration	A. Cultivated field B. Adjacent old-field/pasture	Murray
• 3rd year Burn site	Roadside prairie planting 1990	Renville
• Mountain Lake	Roadside prairie planting 1987	Cottonwood

Table 2. VA mycorrhizae spores per gram dry soil from various prairie locations.

Spore Type	Location						
	Crosstown Prairie	Crosstown-Highway	Feder Prairie	Fuller Lake Rest Area	Materials and Research Lab	Materials and Research Lab RailRoad	University of Minnesota
Large Brown	4.95 ±2.05	4.61 ±0.56	13.76 ±6.16	2.89 ±1.26	3.52 ±0.86	11.00 ±7.39	3.42 ±1.72
Small Brown	8.55 ±2.77	34.86 ±4.65	18.21 ±12.71	58.71 ±16.42	24.29 ±6.85	141.12 ±21.55	13.62 ±2.31
Large Yellow	1.49 ±0.40	6.81 ±0.66	6.75 ±1.71	3.52 ±0.65	11.49 ±3.16	3.14 ±1.21	6.88 ±1.56
Small Yellow	5.93 ±2.50	13.92 ±2.50	10.32 ±3.12	104.21 ±31.57	11.27 ±2.72	47.12 ±17.20	10.74 ±3.14
Large Hyaline	0.12 ±0.08	0.12 ±0.08	0.65 ±0.48	0.04 ±0.04	1.00 ±0.42	0.44 ±0.18	0.40 ±0.35
Small Hyaline	3.73 ±0.69	12.62 ±2.76	5.73 ±2.10	21.09 ±5.64	8.07 ±3.33	8.29 ±1.92	28.52 ±13.56
Large Red	0.00 ±0.00	0.28 ±0.12	0.56 ±0.56	0.68 ±0.54	0.26 ±0.13	8.13 ±3.39	6.49 ±1.98
Small Red	0.35 ±0.19	0.08 ±0.05	0.29 ±0.19	2.72 ±0.98	0.07 ±0.07	11.45 ±4.70	0.52 ±0.39
Black	0.19 ±0.10	1.20 ±0.38	1.74 ±0.49	0.00 ±0.00	0.40 ±0.23	1.55 ±1.12	0.94 ±0.35
Total	25.32 ±3.64	74.48 ±6.80[a]	58.02 ±24.67[a]	193.85 ±32.71[b]	60.37 ±6.44[a]	232.24 ±40.93[b]	71.54 ±16.08[a]

Spores are classified on size (Small: ≤120µm, large ≥130) and color:

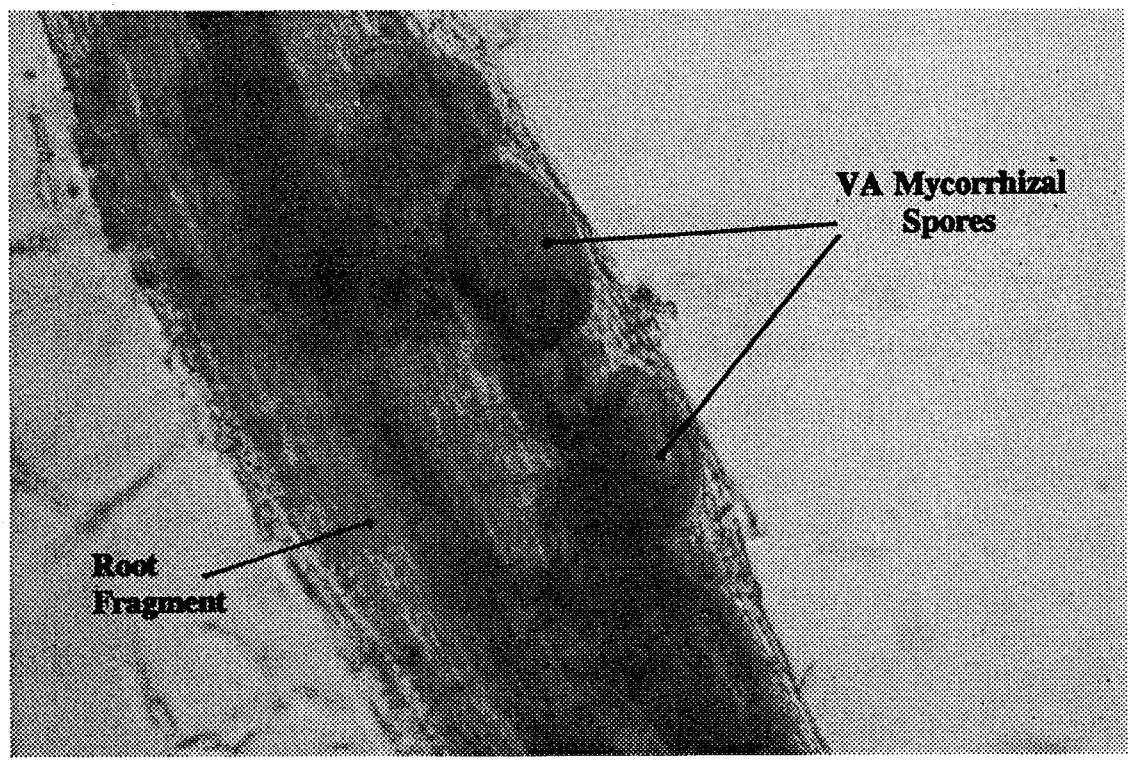
Large-brown spore type: *Entrophospora infrequens* (Hall) Ames & Schneider; *Glomus geosporum* (Nicol. & Gerd.) Walker
Small-brown spore type: **Glomus aggregatum* Schenck & Smith; **Glomus etunicatum* Becker & Gerdemann (Fig. 8)
Large-yellow (≥150µm) spore type: *Gigaspora gigantea* (Nicol. & Gerd.) Gerdemann & Trappe; *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe
Small-yellow (≤120µm) spore type: **Glomus macrocarpum* Tul. & Tul.; **Glomus etunicatum* Becker & Gerdemann; *Glomus fasiculatum* (Thaxter) Gerd. & Trappe emend. Walker & Koske; *Glomus* sp. (unidentified)
Large-hyaline (clear, white or greenish) spore type: *Gigaspora abilda* Schenck & Smith emend. Koske; **Gigaspora decipiens* Hall & Abbott
Small-hyaline spore type: *Glomus albidum*; *Glomus* sp. (unidentified)
Large-red spore type: *Glomus geosporum* (Nicol. & Gerd.) Walker
Small-red spore type: *Glomus* sp. (unidentified), probably immature *Gl. geosporum.*
Black spore type: *Glomus geosporum* (Nicol. & Gerd.) Walker

The Crosstown Prairie (CTP) site had similar spore diversity to all survey sites but had significantly fewer total spore numbers than any site sampled. The adjacent disturbed road (CTP-Hwy) site had three times more spores, differing mainly in small brown, yellow and hyaline spore type frequency. *Glomus gigantea* was also three times more abundant. No significant difference in total spore number was observed between the CTP-Hwy, Feder Prairie (FdrP), MRL-RR and the University of Minnesota (UM) sites. However, spore type frequency distributions varied by site, with three of the four sites dominated by small brown spores of *Glomus aggregatum* and *Glomus etunicatum.* The UM site was dominated by small hyaline spores. The FdrP site displayed the most uniform frequency distribution and the greatest diversity of species of all sites examined.

In 1992, three additional sites were examined; a spring collection from Trunk Highway 60 near Mountain Lake (MtnL), a Lincoln County roadway inslope (LC-iR) and a roadside prairie planting along Trunk Highway 19 near Fairfax (3rd year of establishment) that had been burned earlier that spring (BS3). Overall spore numbers at all sites were similar to FdrP but FdrP exhibited higher VA mycorrhizae species diversity. The prescribed burn at BS3 did not appear to damage mycorrhizal spore number or diversity and is in agreement with the work performed by Bentivenga and Hetrick [17], Vilarino and Arines [8], and Jasper et al. [18].

It was apparent from our survey that excellent VA mycorrhizae spore material is available in remnant Minnesota prairies and that inoculum containing hyphal fragments and spores (Figure 2) can be obtained from soil cores from such areas. Differences between sites due to morphometry, solar orientation and physical location to environmental and atmospheric inputs may reflect the differences observed in mycorrhizal populations; especially at disturbed or recently restored sites. There also appears to be a relationship between vegetative cover (including composition and disturbance) and the types of VA mycorrhizae present. For example, Feder Prairie (a managed remnant containing a high diversity of prairie species) contained the highest diversity and most uniform distribution of VA mycorrhizae spore types. We also observed that the cultivated field at the Lake Shetek site contained a predominance of VA mycorrhizae types that are associated with corn and soybeans and little or no VA mycorrhizae species associated with prairie species.

Figure 2. Mycorrhizal spores on a colonized root



Soil type and fertility may be inversely related to VA mycorrhizae quantities found at different sites. For instance, the Fuller Lake Rest Area planting and the abandoned railroad right-of-way near the Mn/DOT Materials & Research Lab contained greater quantities of spores than did Feder Prairie, but both also contained noticeably poorer soils. This finding is consistent with the hypothesis that VA mycorrhizae associations are more important to species growing in nutrient poor soils than in fertile soils. The heavily disturbed sites at the Crosstown highway and the Materials Lab may contain VA mycorrhizae species that are adapted for disturbed or polluted soils.

At this time no recommendation can be made for matching inoculum source sites with sites to be restored. More work is needed to correlate inoculum source to replanting requirements. We chose to use Feder Prairie and Crosstown Prairie soils for inoculum in Phase 2 because of Feder Prairie's high VA mycorrhizae diversity and Crosstown Prairie's exposure to highway run-off and high degree of disturbance.

CHAPTER 3. PROJECT PHASE 2: VA MYCORRHIZAE INOCULUM PRODUCTION

3.1 Phase 2 Overview

VA mycorrhizae inoculum production either as spores and/or hyphal/root fragments can be expensive and time consuming because VA mycorrhizae must be cultured on living plant hosts. We were interested in setting up a plant system for spore production that used a minimum of greenhouse space and materials. Secondly, the plant growth system had to operate with minimum of maintenance over the three month growing period. Lastly the production system had to be of practical use for future restoration operations performed by Mn/DOT.

We chose native warm-season grasses as mycorrhizal hosts (especially little bluestem) because of their dependence on mycorrhizae for growth and development [6]. This section describes the method developed for low cost, low-maintenance production of mycorrhizal inoculum which could be installed from seed boxes of a Truax native grass seed drill. In this phase of the project we also wished to compare production of inoculum from two source locations; one a high quality prairie remnant and the other a disturbed prairie remnant. Finally we wished to evaluate inoculum production using two different plant hosts, little bluestem (*Schizachyrium scoparium*) and sideoats grama (*Bouteloua curtipendula*).

3.2 Materials and Methods

3.2.1 Germination procedure of little bluestem and sideoats grama

Seeds were placed in trays with a 2 cm layer of University of Minnesota (UM) Greenhouse soil mix, followed by a 1 cm layer of 1:1 mixture of coarse vermiculite (Strong-Lite Products Corp., Shipyard-Road, Seneca, IL 61360) and coarse perlite (Midwest Perlite Co., 4280 W. Parkway Blvd., Appleton WI 54915), seeds and a 1 cm layer of the 1:1 vermiculite/perlite mixture. A plastic cover was placed over the trays which were then placed on a heat pad set at 70-75°F. After 5 days on the heating pad, the plastic covers were removed and the trays were placed under high intensity sodium lights. Seedlings were watered as needed until they were transplanted into experimental pots.

3.2.2 Inoculum Production

Soil samples from Feder Prairie (collected 15 Nov, 1991) and the Crosstown Prairie (collected 14 Nov, 1991) were used as VA mycorrhizae inoculum source. After collection, the soil samples were air dried at room temperature for 7 days and then stored at 5°C for 4-5 weeks to expose VA mycorrhizae spores to vernalization [19, 20].

In January 1992, fifty experimental pots containing Feder Prairie inoculum and 20 experimental pots containing Crosstown Prairie inoculum were planted with 30 day old little bluestem and sideoats grama seedlings. Experimental pots were layered with VA mycorrhizae inoculum soil so that seedling roots would grow down through the VA mycorrhizae layer and contact hyphal fragments and/or spores. Three pots each of little bluestem and sideoats grama were planted without VA mycorrhizae soil layering to serve as controls. Seedlings were grown for 90 days under high intensity growth lights in a University of Minnesota greenhouse and watered once weekly with a 10% Hoagland nutrient solution [21], (see Appendix G). After 90 days watering was terminated and the plants were allowed to senesce for 30 days to maximize spore production.

Soil from the experimental pots was passed through a 2mm sieve to remove large inorganic fragments and to grind up the resulting root mass. The soil was then transported to the Lake Shetek site. Microscopic analysis (as previously described in Phase 1) was performed on samples from experimental and control pots to evaluate colonization and VA mycorrhizae production.

3.3 Results & Discussion

Once the growing system was set up, little maintenance was required to keep it running. Watering and nutrient feeding operations were relatively simple and required little time to perform. We estimate that there was a cost of $0.10 /1000 spores produced with this system. A new hydroponics method employing computerized drip feeding [22] may require even less maintenance and should be considered for future mycorrhizal production projects.

The VA mycorrhizae production system (Figure 3) increased spore numbers by 90 to 363 times, depending on host plant and source inoculum (Table 3). Our spore yields are similar to those reported by other researchers [23, 24]. Greatest spore yields were obtained with little bluestem host plants and Feder

Prairie inoculum. A decrease in large sized spores was observed in nearly all treatments. Apparently production of smaller sized spores of *Glomus aggregatum* and *Glomus albidum* was favored over production of the larger sized spores (e.g. *Glomus mosseae*, *Glomus geosporum*, *Gigaspora gigantea*) under our culture conditions. The only large sized spore that increased was *Glomus albida* grown with little bluestem as host and Crosstown Prairie inoculum.

Figure 3. Pot set-up for VA mycorrhizae production

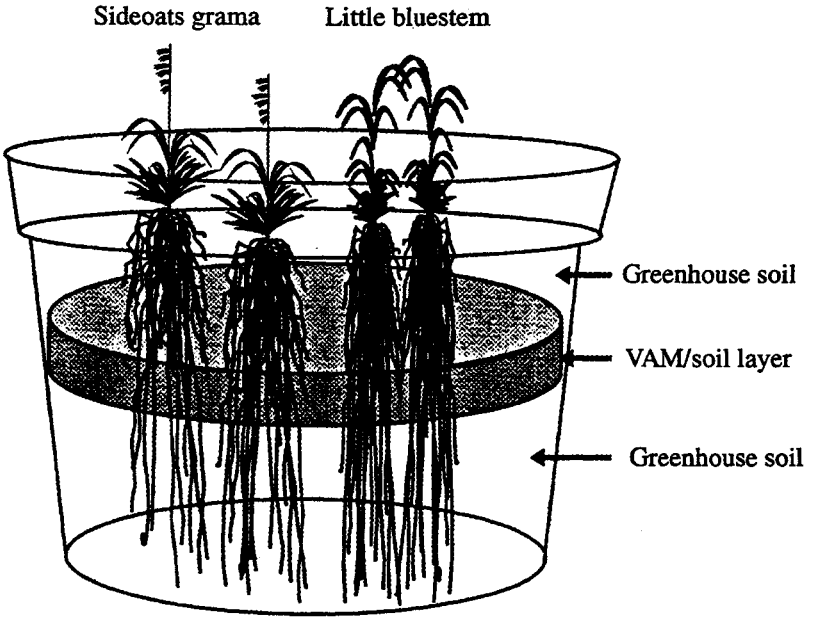


Table 3. Spore number per gram in greenhouse pots at t=0 and t=13 weeks.

Inoculum Source	Host Plant	Spore Type	Spore Number per gram		Spore (x fold) Increase	Frequency		
			Initial	Final		Initial	Final	Percent Change
CTP	LB	Brown	0.052	6.0	115	53.3	28.4	**-46.7**
		Yellow	0.028	6.0	210	29.3	28.4	**-3.1**
		Hyaline	0.015	8.0	540	15.2	37.9	**149.3**
		Reddish /Black	0.002	1.0	538	2.1	5.3	**148.5**
		Total	0.097	21.0	**217**	100.0	100.0	
CTP	SOG	Brown	0.052	7.9	153	53.3	35.9	**-32.7**
		Yellow	0.028	1.5	54	29.3	7.0	**-76.1**
		Hyaline	0.015	11.9	804	15.2	53.9	**254.5**
		Reddish /Black	0.002	0.7	340	2.1	3.2	**50.0**
		Total	0.097	22.0	**227**	100.0	100.0	
FdrP	LB	Brown	0.123	10.0	81	55.1	12.3	**-77.7**
		Yellow	0.066	32.8	500	29.4	40.5	**37.7**
		Hyaline	0.025	37.2	1516	11.0	45.9	**317.4**
		Reddish /Black	0.010	1.1	106	4.5	1.3	**-70.9**
		Total	0.224	81.1	**363**	100.0	100.0	
FdrP	SOG	Brown	0.123	9.5	77	55.1	47.4	**-14.0**
		Yellow	0.066	4.3	66	29.4	21.5	**-26.9**
		Hyaline	0.025	5.8	235	11.0	28.8	**161.9**
		Reddish /Black	0.010	0.5	46	4.5	2.3	**-48.5**
		Total	0.224	20.1	**90**	100.0	100.0	

Final inoculum concentration reflects spore numbers only. A bioassay is recommended to determine the inoculum potential. LB = little bluestem, SOG = sideoats grama.

No mycorrhizal nutrient and energy transfer structures, i.e., vesicles or arbuscules, were observed in either little bluestem or sideoats grama grown with Crosstown Prairie inoculum, whereas these structures were observed in the treatments containing Feder Prairie inoculum. Increased spore production in the Feder Prairie treatments and the observed VA mycorrhizae activity suggest that energy transfer from plant to fungus occurred in those treatments.

Little bluestem plants had higher rates of mycorrhizal activity and root colonization than did sideoats grama in both Feder Prairie and Crosstown Prairie inoculum treatments (Table 4). Although we did not collect data on root masses, we did observe that sideoats grama tended to produce a larger root mass than little bluestem. The VA mycorrhizae activity and root mass observations may indicate that sideoats grama is less dependent than little bluestem on mycorrhizal colonization for soil exploration and

nutrient uptake. In terms of root colonization and spore production, little bluestem represents the better choice of host plant.

Table 4. Percent root colonization/activity of VA mycorrhizae host plants

Host Plant	Inoculum Source	% Root/ VA mycorrhizae Colonization*	% VA mycorrhizae Activity**	Total	# of Root Intersects per gram wet root
Little Bluestem	Feder Prairie	3.94 ±1.01	4.44 ±1.42^{c}	8.38 ±2.31^{a}	335 ±39^{d}
	Crosstown Prairie	0 ±0^{b}	20.35 ±6.40	20.35 ±6.40	469 ±39de
	Control (None)	0 ±0^{b}	0 ±0^{c}	0 ±0^{a}	362 ±78^{d}
Sideoats Grama	Feder Prairie	1.63 ±0.61	2.06 ±0.76^{c}	3.69 ±1.35^{a}	366 ±36^{d}
	Crosstown Prairie	0 ±0^{b}	0 ±0^{c}	0 ±0^{a}	608 ±61^{e}
	Control (None)	0 ±0^{b}	0 ±0^{c}	0 ±0^{a}	631 ±220^{e}

Plants were grown in the Biological Sciences Greenhouse, University of Minnesota for 13 weeks. Numbers listed are averages ±SE.

* Consists of internal mycorrhizal structures, ie. vesicles
** Consists of internal and external mycorrhizal hyphae

The presence of *Gigaspora* species in the Crosstown Prairie treatments may be related to the disturbed nature of that site resulting from salt run-off, etc. The low spore yields with mycorrhizal dependent little bluestem grown with Crosstown Prairie inoculum may indicate incompatible spore ecotypes or species for optimum production. It should be noted that little bluestem is not a large component of the Crosstown Prairie. This suggests the importance of producing inoculum from spore populations near the areas of a prairie restoration.

CHAPTER 4. PROJECT PHASE 3: MYCORRHIZAE INTRODUCTION & MONITORING

4.1 Overview

It has been documented by a number of researchers that mycorrhizal fungi may play an important role in the functioning of prairie ecosystems [2, 3] and greenhouse experiments further indicate that prairie plants benefit from symbiotic associations with VA mycorrhizae [5, 6]. However, little work has been done examining the re-introduction of VA mycorrhizae as a component of a prairie restoration on a large scale. Our objectives for Phase 3 were to:

1. Determine the feasibility of mycorrhizal introduction to soils using current seeding methods,
2. Determine whether re-introduced VA mycorrhizae colonized the roots of recently seeded prairie species.

4.2 Materials and Methods

4.2.1 Study Site.

The field study was conducted at the Lake Shetek wetland mitigation site in Murray County, Minnesota, where 135 acres consisting of tilled farmland and old-field was purchased by the Minnesota Department of Transportation (Appendix C). The site will eventually be transfered to the Minnesota Department of Natural Resources and will become a Wildlife Management Area. Approximately 70 acres of the Shetek site were restored to a wetland state by building a control structure and raising existing water levels. Sixty five acres of upland were restored to native prairie. Prior to acquisition by Mn/DOT, the tilled land had been under a soybean/corn rotation except the last year of agricultural use when wheat had been planted. Soils were high in silt, clay, and organic matter, with a pH of 6.5. Soils were also high in N,P,K, Zn, Fe, Mn, Cu, B, Ca and Mg (see Appendix F).

4.2.2 Introduction of VA mycorrhizae to Field Plots

Mycorrhizal spores and colonized root fragments from 9 replicate experimental pots produced in Phase 2 were mixed thoroughly after passing through a 2 mm sieve. Large root and other organic and inorganic materials were discarded after rubbing on the sieve surface to dislodge loose soil fragments. The resulting soil/inoculum preparation was stored at room temperature in large, unsealed plastic bags until transport to the field site. Zones corresponding to each treatment extend lengthwise 0.25 miles

down the farm field in the direction that the seeder was driven (orientation is generally north to south). The width of each zone varied from 7 to 25 meters. The width of the zones was limited by time and seed box emptying rate.

Seed and inoculum was installed using a proto-type interseeder drill which was on loan for demonstration purposes from Jim Truax. The drill contained three seed boxes; one for fine seeds, one for fluffy seeds and a grain box. The drill was set up so that fine seeds were dropped onto the soil surface from the fine seed box and seed in the fluffy and grain boxes was placed approximately 1/2 inch deep into the soil. Seed and soil/VA mycorrhizae inoculum and corresponding to the different treatment zones was installed from the seed drill as follows:

Treatment (Zone)	VA mycorrhizae Inoculum Quantity
Buffer:	no VA mycorrhizae added to seeder.
Low Density:	9 pot quantity was added to the fluffy seed box.
High Density:	18 pot quantity total (9 pot quantity in the fluffy seed box with the native grasses, and 9 pots in the grain seed box containing oats).
Residual:	Majority of High Density inoculum consumed in plot with residual inoculum remaining in seed box.

4.2.3 Field Monitoring: Sample Area Set-up and Monitoring

After treatment zone boundaries were determined, parallel transects were established down the center of each treatment zone. Transects began 50m south from a line perpendicular to a USGS survey marker at the edge of the site. Six sampling areas spaced 50m apart along each transect were set up in each treatment zone. Sample areas were marked with a yellow flag so that we could return to the same area throughout the summer. Within a 2m radius from each flag, a 0.25m grid was randomly dropped, all plant counts, soil samples etc., were obtained from within the grid (no two grids were resampled through the course of the season).

Monitoring was conducted at 4 week intervals. Each monitoring consisted of counting shoot numbers and plant type (monocot vs. dicot). If a seedling was identifiable within the angiosperm family,

it was recorded as a subset of the family. Soil temperature was measured with each sample. In July, vegetation at the site was mowed by Mn/DOT maintenance personnel because the oats used as a cover crop had reached a height of 5 feet. Mowing height was approximately six inches. The October analysis included 0.25m destructive harvest for total biomass and identification of plants at the family level.

4.2.4 Colonization and mycorrhizal activity in roots.

The presence of mycorrhizal fungi inside roots was assessed at 4 week intervals from July to October 1992. A subsample (approximately 0.25g fresh weight) was stained in Acid fuschin [25] for each sample. The proportion of root with inner or outer mycorrhizal structures was determined by the gridline-intercept method [26].

4.2.5 Statistical analysis.

Analysis of variance was conducted using Statview SE+Graphics, v. 1.03 (Abacus Concepts, Inc., 1988). Percent colonization data were log(1+x) transformed [16] to normalize the data set prior to analysis. Mean separation was determined using the least significant difference (LSD) criteria.

4.3 Results & Discussion

4.3.1 Installation of Seed and Inoculum

The inoculum preparation fed through the seed drill quite well and the presence of perlite granules in the preparation made it easy to tell whether inoculum was feeding through and being placed in the drill rows with the seed.

4.3.2 Plant Germination and Survival

High variability in the number of plants that germinated and survived in the four zones was observed. Mowing the site in July had a detrimental affect on dicots present and had little affect on monocots. Dicots showed a recovery only in the Low Density (LD) zone after being mowed. Monocot numbers were low in the LD zone throughout the summer (Tables 5a & b). The total number of plants in October was approximately 1200/m^2 and did not differ significantly in the four zones (Table 5a). No identifiable prairie plant was higher than 30 cm in October.

4.3.3 Biomass

Plant biomass did not differ significantly in the four zones (Table 5a). The highest percent error of the means (variability) was in the buffer zone.

Table 5a. Plant counts and biomass at the Lake Shetek field site.

	Plant Number/m^2				Biomass (g/m^2)			
Zone	**Monocots[1]**	**Dicots**	**Oats**	**Total**	**Monocots[1]**	**Dicots**	**Oats[2]**	**Total[3]**
Buffer	797 ±201[a]	349 ±91[c]	310 ±137[d]	1456 ±65[e]	221 ±75[f]	286 ±104[g]	100 ±50[h]	507 ±140[i]
Low Density	374 ±78[b]	634 ±95	42 ±27	1050 ±144[e]	94 ±25[f]	350 ±58[g]	13 ±8[h]	444 ±62[i]
High Density	618 ±69[ab]	365 ±47[c]	224 ±152[d]	1206 ±175[e]	188 ±49[f]	249 ±73[g]	58 ±40[h]	437 ±84[i]
Residual	589 ±144[ab]	352 ±37[c]	221 ±56[d]	1162 ±182[e]	138 ±30[f]	195 ±34[g]	61 ±16[h]	333 ±17[i]

Final plant counts were taken at the end of the growing season within the field transects in each mycorrhizal zone from October 1992 in the Lake Shetek mitigation study area. The buffer zone had no mycorrhizal inoculum added. Data below are means (n=5) ± SE. Means within the columns followed by identical letters are not significantly different at the 5% level from One-factor ANOVA analysis. [1]Does not include oats. [2]Oats were cut in July to allow photo energy to penetrate the canopy. The biomass reported above represent the stubble remaining. This column is for comparison between sites only. [3]Does not include oat biomass (not available to measure due to midseason cutting).

Table 5b. Plant number* per m^2 in the Lake Shetek study area.

	Zone			
Monocots*	**Buffer**	**Low Density**	**High Density**	**Residual**
6-Jul-92	121 ±36	65 ±18	118 ±30	
4-Aug-92	187 ±56	101 ±32	114 ±37	196 ±71
8-Sep-92	340 ±80	172 ±17	273 ±61	327 ±91
8-Oct-92	797 ±201	374 ±78	618 ±69	589 ±144
Dicots	Buffer	Low Density	High Density	Residual
6-Jul-92	771 ±55	799 ±47	661 ±145	
4-Aug-92	340 ±39	439 ±60	286 ±43	235 ±36
8-Sep-92	284 ±83	459 ±32	314 ±40	230 ±32
8-Oct-92	349 ±91	634 ±95	365 ±47	352 ±37

* Number does not include the oats cover crop.

4.3.4 Mycotrophy

In general, the highest VA mycorrhizae activity occurred in midsummer (August 4) and declined through October. This corresponded to increasing root colonization by mycorrhizae over the growing

season. In October, no significant difference was observed in any zone in VA mycorrhizae root activity and colonization (2% and 7% respectively, see Table 6).

Table 6. Percent VA mycorrhizae activity and root colonization.

6a. % VA mycorrhizae Activity Date (1992)	Zone			
	Buffer	**Low Density Inoculum**	**High Density Inoculum**	**Residual**
July 6	0.86 ± 0.51^{a}	1.30 ± 0.62^{ab}	4.53 ± 1.45^{ce}	NC
August 4	2.93 ± 0.80^{ac}	4.84 ± 1.59^{c}	4.52 ± 1.40^{ce}	3.96 ± 1.21^{bcd}
September 8	1.33 ± 0.30^{ad}	2.04 ± 0.35^{ac}	1.78 ± 0.37^{ac}	3.87 ± 2.42^{ac}
October 8	2.15 ± 0.73^{ac}	2.35 ± 0.95^{ac}	1.28 ± 0.22^{ad}	1.55 ± 0.33^{ade}
6b. % Root Colonization				
July 6	1.07 ± 0.15^{vz}	2.00 ± 1.04^{vyz}	3.42 ± 0.92^{vxz}	NC
August 4	0.57 ± 0.19^{v}	1.94 ± 1.15^{tvz}	5.28 ± 1.96^{svz}	3.19 ± 2.38^{uvz}
September 8	5.26 ± 1.71^{svz}	2.41 ± 1.31^{rtvz}	5.31 ± 2.01^{svz}	6.32 ± 1.96^{stuxy}
October 8	7.38 ± 2.11^{sux}	6.37 ± 2.57^{stuxy}	5.67 ± 2.07^{uxyz}	7.13 ± 1.70^{rsux}

VA mycorrhizae activity is the number of fungal structures outside the root per unit root length and root colonization is number of fungal structures within the root per unit root length. Data are means ± SE. Data within rows and columns followed by an identical letter are not significantly different at the 5% level in one-factor ANOVA analysis after log(1-x) transformation. NC = roots not collected for analysis.

4.3.5 VA mycorrhizae Spore Populations Prior to Seeding

The highest number of recovered spores occurred in May, prior to any mycorrhizal additions. Total spore number was the same in the tilled field and the adjacent pasture. However, the frequency distribution between the two sites differed in the number of small brown and large yellow spore types (Table 7). The field collection of June immediately prior to planting and spore placement, shows naturally occurring spore frequency and total number differences in the treatment zones. The area designated as High Density (HD) displayed half the number of total spores as the buffer and low density zones. There were significant differences between spore types of small brown, large & small yellow and small hyaline.

Table 7. VA mycorrhizae spore number from Lake Shetek wetland mitigation/prairie restoration site per gram dry soil.

Spore Color	Preseed: 13 May 1992 Recently Plowed	Preseed: 13 May 1992 Adjacent Pasture	Preseed: 4 June 1992 B	Preseed: 4 June 1992 LD	Preseed: 4 June 1992 HD	6 July 1992 B	6 July 1992 LD	6 July 1992 HD	11 November 1992 B	11 November 1992 LD	11 November 1992 HD	11 November 1992 R
Large Brown	9.0 ± 2.8^{a}	9.8 ± 3.1^{a}	3.9 ± 1.4^{bc}	4.6 ± 1.5^{abc}	1.4 ± 0.3^{c}	7.4 ± 1.6^{ab}	3.2 ± 1.7^{bc}	3.8 ± 1.3^{bc}	4.5 ± 0.7^{ab}	3.2 ± 0.1^{bc}	4.8 ± 0.8^{ab}	1.2 ± 0.3^{c}
Small Brown	96.6 ± 18.6^{d}	16.2 ± 4.2^{ef}	25.9 ± 6.7^{f}	14.4 ± 3.2^{ef}	6.9 ± 2.0^{e}	15.0 ± 3.4^{ef}	18.5 ± 9.8^{ef}	14.2 ± 5.0^{ef}	35.7 ± 5.4^{df}	15.8 ± 8.6^{ef}	28.5 ± 9.8^{f}	10.5 ± 1.2^{ef}
Large Yellow	5.9 ± 1.3^{gij}	25.4 ± 7.3^{h}	5.0 ± 0.8^{gij}	3.6 ± 1.2^{gjk}	3.8 ± 1.3^{gjk}	3.3 ± 0.6^{gj}	3.0 ± 1.7^{g}	3.5 ± 0.7^{gjk}	16.0 ± 3.6^{hl}	7.6 ± 1.6^{ijl}	10.3 ± 1.3^{hi}	14.0 ± 9.4^{hik}
Small Yellow	33.5 ± 6.4^{mn}	50.0 ± 16.3^{n}	22.7 ± 4.9^{mno}	17.1 ± 6.1^{mp}	7.5 ± 2.5^{p}	12.5 ± 3.0^{op}	14.9 ± 6.6^{op}	8.0 ± 1.2^{p}	13.0 ± 1.6^{op}	11.2 ± 2.6^{op}	15.3 ± 2.6^{mop}	14.3 ± 2.6^{mop}
Lrg Hyaline	0.1 ± 0.1^{q}	0.3 ± 0.2^{q}	0.1 ± 0.1^{q}	0.1 ± 0.1^{q}	0.02 ± 0.02^{q}	0^{q}	0^{q}	0^{q}	0^{q}	0^{q}	0^{q}	0^{q}
Sm Hyaline	7.5 ± 1.3^{rs}	25.4 ± 10.9^{s}	5.3 ± 1.0^{r}	2.1 ± 0.6^{rt}	1.2 ± 0.6^{tu}	1.3 ± 0.5^{tu}	1.5 ± 1.5^{tu}	0.7 ± 0.3^{t}	4.2 ± 1.2^{ru}	0.6 ± 0.1^{t}	14.1 ± 4.5^{rs}	1.1 ± 1.0^{tu}
Large Red	0.2 ± 0.1^{v}	0.5 ± 0.2^{vw}	0.5 ± 0.3^{vw}	1.2 ± 0.5^{w}	1.5 ± 0.7^{w}	0.2 ± 0.1^{vw}	0.3 ± 0.2^{vw}	1.7 ± 0.9^{w}	4.9 ± 1.7^{x}	7.9 ± 0.6^{x}	6.8 ± 2.5^{x}	3.8 ± 0.8^{x}
Small Red	0^{α}	$0.1 \pm 0.1^{\alpha}$	$2.5 \pm 1.5^{\alpha\beta}$	$1.5 \pm 1.4^{\alpha\beta}$	$2.4 \pm 2.4^{\alpha\beta}$	$0.04 \pm 0.04^{\alpha}$	0^{α}	0^{α}	0^{α}	0^{α}	$0.3 \pm 0.3^{\alpha\beta}$	0^{α}
Lrg. Black	$0.4 \pm 0.3^{\gamma}$	0^{α}	$0.1 \pm 0.1^{\alpha}$	$0.1 \pm 0.1^{\alpha}$	0^{α}	$0.06 \pm 0.04^{\alpha}$	$0.1 \pm 0.1^{\alpha}$	0^{α}	$0.3 \pm 0.2^{\alpha\gamma}$	0^{α}	$0.1 \pm 0.1^{\alpha}$	0^{α}
Total	153 ± 18^{y}	128 ± 24^{yz}	66 ± 9^{a}	45 ± 4^{ab}	25 ± 5^{c}	40 ± 7^{bcd}	42 ± 21^{bcd}	32 ± 5^{bcd}	79 ± 4^{ya}	46 ± 8^{ad}	80 ± 15^{za}	45 ± 13^{ac}

Data are listed as means ±SE. Identical superscripted letters within a row indicate no significant difference in one-factor ANOVA after log(1+x) transformation using the LSD test for mean separation set at the 5% level. B = buffer zone, LD = low density zone, HD = high density zone.

4.3.6 VA Mycorrhizal Spore Populations After Seeding

After mycorrhizal spore soil additions, no significant difference could be detected in any zone, from July to November (Table 7; Stenlund personal observation). The final spore number from November was statistically identical to the highest spore number of June (prior to seeding), but only the Buffer and HD zones matched in number to the May collection. No change in total spore number was observed between the preseed and final collection in the Buffer and LD zones. A frequency shift was observed with changes in large yellow and large red spore types. The HD zone had increased 3x over the same period from 25 to 80 spores per gram soil. Most spore types exhibited increased frequency distribution.

A number of factors were probably involved in causing the high variability in germination and plant numbers we observed between treatments throughout the course of the season. We would expect there to be a large number of annual species such as foxtail, mustards etc., to be present because we were planting in a previously cultivated field. In fact, the pre-existing weed seed-bank was probably as great as the amount of seed that we installed.

We also experienced some difficulty with the seed drill during installation. The no-till attachments caused a build-up of soil under the drill which resulted in clogging of the seeding mechanism. Some seed probably never got into the ground and some may have been buried too deep. The situation was rectified by removing the no-till coulters. It should be noted that this was not a fault with the drill, rather it was our fault for using a no-till drill in a tilled field.

Mowing the site in July had a significant affect on the growth of plants in the field plots. However, we felt that mowing was necessary because the cover crop of oats was so tall and dense that it was shading out the developing native grass and forb seedlings (mowing is common in new plantings for this very same reason). Soil fertility also probably had a great affect on the growth of plants in field plots, especially contributing to the vigorous growth of the oats. Soil fertility would be expected to decrease over time as fertilizer inputs decrease and prescribed burns to manage the prairie are implemented.

For this experiment the prairie plant populations were too young and the soil was too fertile to make conclusions concerning the long-term affect of mycotrophy on plant growth and flowering. However, we were not attempting to answer these questions with this project, and it should be noted that variable results such as ours are not unusual in VA mycorrhizae research [23, 24].

Decreases in spore number between the May and June collections could be attributed to spore germination and/or predation by nematodes which were present in large numbers in the soil samples. Frequency shifts in spore types might be due to the changing composition in vegetation at the site. The lack of recovery in spore numbers in the fall may reflect this vegetation shift as well. For example, spores associated with agronomic crops which were present in the spring would be expected to disappear gradually because crops were no longer being planted. A corresponding increase in VA mycorrhizae spores that we introduced with the prairie species would be expected over time, but might not be manifested in our fall collections because of the young age of the planting. It will be interesting to come back to this site in several years to examine spore populations as the prairie matures. A site visit in August, 1993 showed the prairie planting progressing well and the site is scheduled for a burn in the spring of 1995. Soil samples were not taken in 1993.

We feel that this project demonstrated that VA mycorrhizae associated with native prairie species can be cultured and increased in a greenhouse and then be re-introduced back into a prairie restoration. Re-introduced VA mycorrhizae were found to colonize seedlings in the field plots. Specialized equipment was not necessary for VA mycorrhizae re-introduction because the inoculum preparation fed through the seed drill quite well.

In concluding, we would recommend that continued monitoring of the existing plots should be done. We would also recommend that additional plots be established at roadside and non-roadside sites containing less fertile soils. We expect that VA mycorrhizae effects would be more noticeable under harsher conditions. Slight equipment modifications such as decreasing drill row spacing might place inoculum and seed closer together spatially in new sites and increase the chance for colonization.

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APPENDIX A

VA MYCORRHIZAE SPORE ISOLATION PROTOCOL

Appendix A. Spore Isolation Protocol

After complete dry-down of soil, a 10, 15 or 25 gram homogenized sample will be obtained using a Mettler balance (PM600), depending on the type of substrate. Samples high in organic matter require a smaller initial sample size to prevent the filter paper from overloading. The ideal sample weight is 25 grams. Soil samples are placed into plastic 500 ml screw top Erlenmeyer flasks (Nalgene), and the following process is followed:

a. Add ten ml sodium metaphosphate via a Repipet II along with 90 ml deionized water.

b. Attach flask(s) to a mechanical shaker (Burrell Wrist Action Shaker, Model 75) for 15 minutes.

c. Allow shakate to settle for 15 seconds (about the length of time required to release the shaker fingers) before decanting through a series of USA Standard Testing sieves composed of a 250, 90, and 25µm mesh sizes.

d. Resuspended the remaining precipitate in 200 ml deionized water, and agitate for an additional 2 minutes and decant as above. Repeat this spore washing process an additional 2 times.

e. Place the material remaining on the 250µm sieve after rinsing sieve into a labeled small plastic beakers containing Ringer's solution. Examination and count spore after transferring precipitate transferred to an etched petri plate (allowed complete viewing without the possibility of overlapping view lanes).

f. Rinsing the material on the 90 and 25µm sieves, and combine the suspensions. Place into plastic centrifuge tubes via a funnel. Balanced all tubes prior to placement into the centrifuge (IEC HN-SII) and spin at full setting (~2500 RPM) for 4 minutes. Save the supernatant on the small 25µm sieve and examine for spores. Resuspended the precipitate in 30 ml of 2M sucrose (684g/L) and spin at full speed for 2 minutes. Decant the sucrose solution expeditiously in the 25µm sieve and follow by several water rinses to remove all osmotically damaging sucrose.

g. Although the sievate could be transferred to a beaker, I preferred to transfer directly from the small sieve to the gridded 0.22µm membrane filter cellulose on the filter apparatus. I believe less loss of spores though attachment to the funnel and glassware occurs. The filter papers are stored in petri plates in the refrigerator for later examination.

h. Transfer the spores from the petri plates to a Gelman filter paper holder (Gelman Science Inc.) for examination and counting at 400 and 1000 x with a Nikon compound microscope.

i. If the number of spores in a grid is 3 or greater, only 20 contiguous grids need to be counted. Indicate by writing 20^2 after the numerical entry into the lab book; otherwise, the whole grid filter paper needs to be counted with the letter T written after the entry to designate a total count. Transfer filters with an appreciable number of spores to two sections of one slide and label with an accession number as a voucher slide.

APPENDIX B

VOUCHER SLIDES

Appendix B. Voucher Slides On File At UM Plant Biology Department

Accession No.	Site	Transect	Replicate	Collection Date	Date Processed	Description	Step	Notes
2000	Burn Site	Composite	Bulked	13-May-92		Spores	250µ	Photo
2001	Burn Site	Composite	Bulked	13-May-92		Spores		Photo
2002	Crosstown	Little bluestem	C		16-Apr-92	Roots		Pot Inoc Production
2003	Crosstown	Little bluestem	E		22-May-92	Spores		Pot Inoc Production
2004	Crosstown	Little bluestem	A		16-Apr-92	Roots		Pot Inoc Production
2005	Crosstown	Little bluestem	E		16-Apr-92	Roots		Pot Inoc Production
2006	Crosstown	Prairie	4	14-Nov-91	14-Jul-92	Spores	sucrose	
2007	Crosstown	Prairie	4	14-Nov-91	14-Jul-92	Spores	water	
2008	Crosstown	Prairie	3	14-Nov-91	10-Mar-92	Spores		
2009	Crosstown	Prairie	4	14-Nov-91	8-Jul-92	Spores	250µ	
2010	Crosstown	Roadside	1	14-Nov-91	15-Jul-92	Spores	250µ	
2011	Crosstown	Roadside	5	14-Nov-91	17-Jun-92	Spores	water	
2012	Crosstown	Roadside	5	14-Nov-91	17-Jun-92	Spores	sucrose	
2013	Crosstown	Side oats grama	C		13-May-92	Spores		Pot Inoc Production
2014	Crosstown	Side oats grama	E			Roots		Pot Inoc Production
2015	Feder Prairie	Little bluestem	5		16-Apr-92	Roots		Pot Inoc Production
2016	Feder Prairie	Little bluestem	4		16-Apr-92	Roots		Pot Inoc Production
2017	Feder Prairie	Little bluestem	2			Roots		Pot Inoc Production
2018	Feder Prairie	Little bluestem	3			Spore wefts	250µ	
2019	Feder Prairie	Side oats grama	4			Roots		Pot Inoc Production
2020	Feder Prairie	Side oats grama	3			Roots		Pot Inoc Production
2021	Feder Prairie	Side oats grama	5			Roots		Pot Inoc Production
2022	Feder Prairie	Side oats grama	5			Spores		Pot Inoc Production
2023	Feder Prairie		3		21-Jul-92	Spores	250µ	
2024	Fuller Lake	Rest Area	1		26-Jul-92	Spores	250µ	Photo
2025	Fuller Lake	Rest Area	4		29-Jul-92	Spores	sucrose	
2026	Fuller Lake	Rest Area	3		20-Jul-92	Spores	sucrose	
2027	Fuller Lake	Rest Area	2		15-Jul-92	Spores	water	
2028	Fuller Lake	Rest Area	2		18-Jun-92	Spores		Gl. geosporum
2029	Fuller Lake	Rest Area	1		28-Jul-92	Spores	water	
2030	Fuller Lake	Rest Area	1		24-Jul-92	Spores	sucrose	
2031	Fuller Lake	Rest Area	2		15-Jul-92	Spores	sucrose	

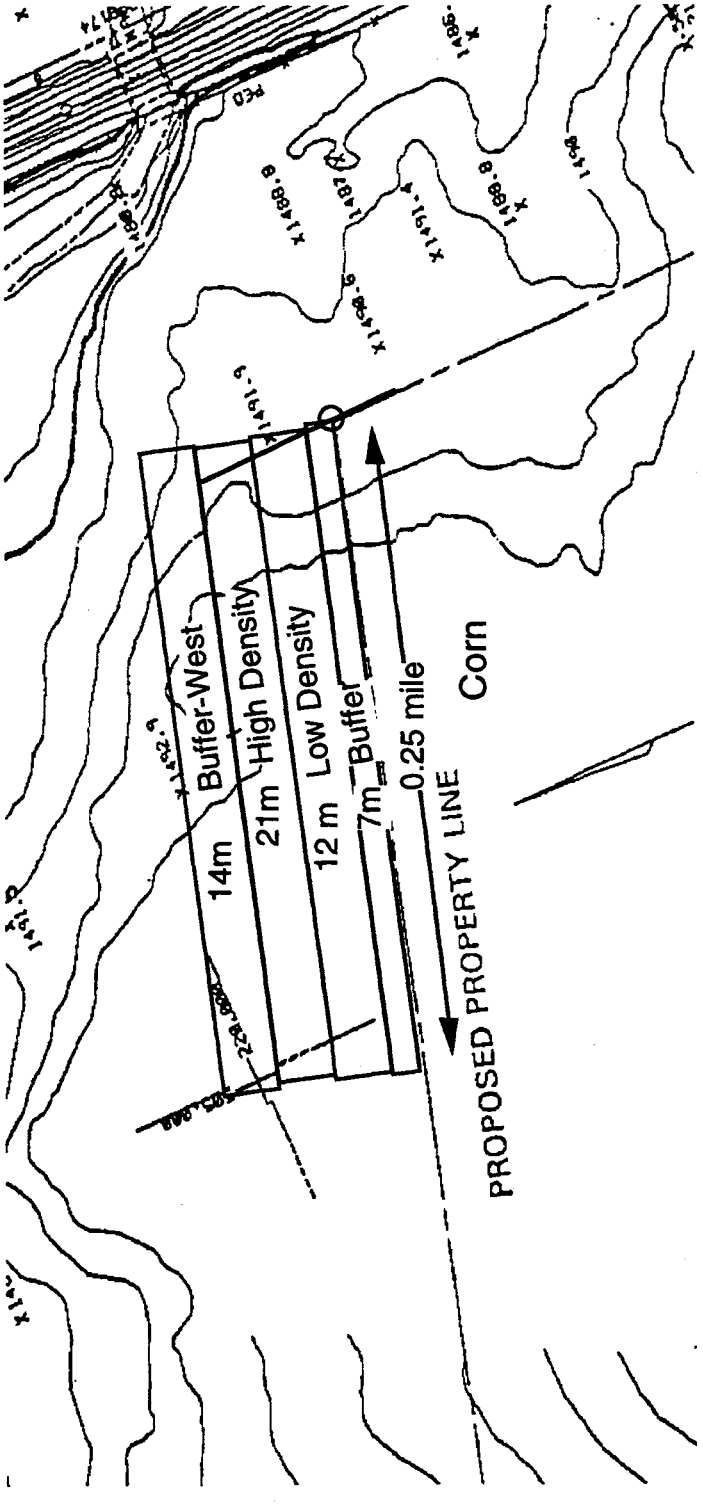
2032	Fuller Lake	Rest Area	3		22-Jul-92	Spores	water	
2033	Fuller Lake	Rest Area	2		17-Jun-92	Spores	250µ	
2034	Fuller Lake	Rest Area	4		25-Jun-92	Spores	250µ	sporocarp
2035	Lake Shetek	Buffer	2	6-Jul-92	24-Jun-92	Spores	250µ	
2036	Lake Shetek	Buffer	1	6-Jul-92	22-Jul-92		250µ	
2037	Lake Shetek	Buffer	2	6-Jul-92		Roots		
2038	Lake Shetek	Buffer	5	6-Jul-92		Roots		
2039	Lake Shetek	Buffer	1	6-Jul-92		Roots		
2040	Lake Shetek	Buffer	3	6-Jul-92		Roots		Photo
2041	Lake Shetek	Buffer	4	4-Aug-92		Roots		Photo
2042	Lake Shetek	Buffer	3	4-Aug-92	27-Aug-92	Spores	250µ	
2043	Lake Shetek	Buffer	3	7-Sep-92	15-Sep-92	Roots		
2044	Lake Shetek	Buffer	5	7-Sep-92	15-Sep-92	Roots		
2045	Lake Shetek	Buffer-West	5	4-Aug-92		Spores	sucrose	Photo
2046	Lake Shetek	Buffer-West	1	4-Aug-92		Roots		Photo
2047	Lake Shetek	Buffer-West	4	4-Aug-92		Roots		Photo
2048	Lake Shetek	Buffer-West	3	7-Sep-92	15-Sep-92	Roots		
2049	Lake Shetek	Buffer-West	5	7-Sep-92	15-Sep-92	Roots		
2050	Lake Shetek	Buffer-West	2	7-Sep-92	15-Sep-92	Roots		
2051	Lake Shetek	Field	2	13-May-92	30-Jun-92	Roots	250µ	
2052	Lake Shetek	Field	C/3	13-May-92	19-May-92	Spores		
2053	Lake Shetek	Field	C/3	13-May-92	19-May-92	Spores		
2054	Lake Shetek	Field	A/1	13-May-92	17-Jun-92	Spores		Photo
2055	Lake Shetek	HD Treatment	4	4-Jun-92		Spores	Sucrose	
2056	Lake Shetek	HD Treatment	2	4-Jun-92		Spore:Giga	250µ	Photo
2057	Lake Shetek	HD Treatment	2	4-Jun-92		Spores		Photo
2058	Lake Shetek	HD Treatment	4	4-Jun-92		Spores	250µ	Photo
2059	Lake Shetek	HD Treatment	5	4-Jun-92		Spores	sucrose	Photo
2060	Lake Shetek	HD Treatment	3	4-Jun-92		Spores	250µ	
2061	Lake Shetek	HD Treatment	5	6-Jul-92		Roots		
2062	Lake Shetek	HD Treatment	4	6-Jul-92		Roots		
2063	Lake Shetek	HD Treatment	3	6-Jul-92		Roots		
2064	Lake Shetek	HD Treatment	4	6-Jul-92	12-Aug-92	Soil animals	sucrose	Photo
2065	Lake Shetek	HD Treatment	2	6-Jul-92		Roots		Photo
2066	Lake Shetek	HD Treatment	3	4-Aug-92		Roots		Photo
2067	Lake Shetek	HD Treatment	4	4-Aug-92		Spores	250µ	Photo
2068	Lake Shetek	HD Treatment	4	4-Aug-92		Spores	sucrose	Photo
2069	Lake Shetek	HD Treatment	1	4-Aug-92		Roots		Photo
2070	Lake Shetek	HD Treatment	2	4-Aug-92		Roots		Photo
2071	Lake Shetek	HD Treatment	4	7-Sep-92	15-Sep-92	Roots		
2072	Lake Shetek	LD Treatment		4-Jun-92	27-Aug-92	spores	38µ	Photo
2073	Lake Shetek	LD Treatment		4-Jun-92		Spores	250µ	
2074	Lake Shetek	LD Treatment	2	6-Jul-92		Roots		
2075	Lake Shetek	LD Treatment	4	6-Jul-92		Spores	sucrose	
2076	Lake Shetek	LD Treatment	5	6-Jul-92		Roots		
2077	Lake Shetek	LD Treatment	4	6-Jul-92		Roots		
2078	Lake Shetek	LD Treatment	4	4-Aug-92		Roots		Photo
2079	Lake Shetek	LD Treatment	2	4-Aug-92		Roots		
2080	Lake Shetek	Mixture		7-Sep-92	15-Sep-92	Roots		

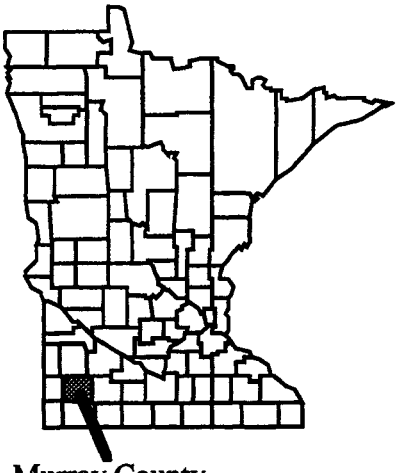
2081	Lake Shetek	Pasture	A	13-May-92	17-Jun-92	Spores		
2082	Lake Shetek	Pasture	C	13-May-92	21-May-92	S/Rt frag	250µ	
2083	Lake Shetek	Pasture	C	13-May-92	21-May-92	Spores		
2084	Lake Shetek	Pasture	C	13-May-92	21-May-92	Spores		
2085	Lake Shetek	Preseed	4	4-Jun-92	2-Jul-92	Spore:Giga	250µ	Photo
2086	Lake Shetek		mixture	6-Jul-92		Roots		
2087	Lake Shetek		5		1-Jul-92	Spores	250µ	
2088	Materials & Research Lab.	Plateau	2	14-Nov-91	14-Jul-92	Spores	250µ	Photo
2089	Materials & research Lab.	Plateau	1	14-Nov-91	15-Jun-92	Spores	250µ	
2090	Materials & research Lab.	Plateau	5	14-Nov-91	18-Jun-92	Spores	250µ	
2091	Materials & research Lab.	Plateau	4	14-Nov-91		Spores	250µ	
2092	Materials & research Lab.	Plateau	2	14-Nov-91	16-Jul-92	Spores		
2093	Materials & research Lab.	Plateau	3	14-Nov-91		Spores	250µ	
2094	Materials & research Lab.	Plateau	5	14-Nov-91	18-Jun-92	Spores	sucrose	
2095	Materials & research Lab.	RailRoad	3	14-Nov-91	7-Aug-92	Spores	250µ	sporocarp
2096	Materials & research Lab.	RailRoad	4	14-Nov-91	7-Aug-92	Spores	250µ	
2097	Materials & research Lab.	RailRoad	1	14-Nov-91		Spores	250µ	
2098	Materials & research Lab.	RailRoad	2	14-Nov-91		Spores		
2099	Mountain Lake	Composite	Bulked	13-May-92	23-May-92	Spores	250µ	Photo
2100	Mountain Lake			13-May-92	23-May-92	Spores	250µ	
2101	University of Minnesota	Roadside	2	15-Nov-91	20-Jun-92	Spores	250µ	
2102	University of Minnesota	Roadside	5	15-Nov-91	23-Jun-92	Spores	sucrose	
2103	University of Minnesota		1	15-Nov-91	21-Jul-92	Spores	250µ	Photo

APPENDIX C

LOCATION OF STUDY SITE

Appendix C. Map of Lake Shetek study site, Murray County, Minnesota.





APPENDIX D

PERCENT COLONIZATION

Appendix D. VA mycorrhizae activity and root colonization in field plots

Transect	Date	Percent Root VA mycorrhizae Activity[1]	Percent Root VA mycorrhizae Colonization[2]
	June 4	0	0
Buffer (Zero VA mycorrhizae Input)	July 6	0.86 ± 0.51^{a}	1.07 ± 0.15^{df}
	August 4	2.93 ± 0.80^{bc}	0.57 ± 0.19^{fh}
	September 8	1.33 ± 0.30^{ab}	5.26 ± 1.71^{egij}
	October		
Buffer-West (Zero VA mycorrhizae Input)	July 6	nc	nc
	August 4	3.96 ± 1.21^{c}	3.19 ± 2.38^{dhi}
	September 8	3.87 ± 2.42^{bc}	6.32 ± 1.96^{g}
	October		
Low Density VA mycorrhizae Input	July 6	1.30 ± 0.62^{ab}	2.00 ± 1.04^{de}
	August 4	4.84 ± 1.59^{c}	1.94 ± 1.15^{dh}
	September 8	2.04 ± 0.35^{abc}	2.41 ± 1.31^{dhj}
	October		
High Density VA mycorrhizae Input	July 6	4.53 ± 1.48^{c}	3.42 ± 0.92^{dg}
	August 4	4.52 ± 1.40^{c}	5.28 ± 1.96^{dg}
	September 8	1.78 ± 0.37^{abc}	5.31 ± 2.01^{egi}
	October		

The data listed are written as the mean ± standard error of 5 replicates. One factor ANOVA analyses were performed after log(1+x) transformation using the Least Significant Differences test set at the 5% level.

[1]: VA mycorrhizae activity is defined by the observation of fungal structures **outside** a root and is calculated by counting the single point episodes of extramatrical hyphae and spores adjacent to a root per intersect grid (x 100).
[2]: Percent root colonization is the number of fungal structures **within** a root of a known length (x 100).

APPENDIX E

SOIL TEMPERATURE

Appendix E. Soil temperatures at the Lake Shetek study site

Date 1992	Zone	Soil Temp ^{0}C
6 July	Buffer	18.5 ±0.2
	Low Density	17.6 ±0.5
	High Density	19.6 ±0.3
4 August	Buffer	18.2 ±0.2
	Low Density	18.5 ±0.5
	High Density	18.5 ±0.4
	Residual	18.0 ±0.4
8 September	Buffer	13.8 ±0.2
	Low Density	14.9 ±0.4
	High Density	15.2 ±0.2
	Residual	14.5 ±0.3
8 October	Buffer	7.4 ±0.3
	Low Density	7.6 ±0.3
	High Density	7.9 ±0.2
	Residual	7.9 ±0.2

APPENDIX F

SOIL MINERAL ANALYSIS

Appendix F. Soil mineral analysis (collected May 1993).

Test	Value	Interpretation
Soil Texture	Medium (loam, silt loam)	
Organic matter	6.8%	High concetration
Soluble salts (mmhos/cm)	0.3	Low, no problem
pH	6.2	slightly acidic
Nitrate (NO_3-N) ppm	21	Very High
Bray 1 P (ppm)	39	Very High
Potassium	300+	Very High
Sulfur SO_4-S	4	Low
Zinc	2.8	Very High
Iron	99.9+	Very High
Manganese	50.8	Very High
Copper	1.5	Very High
Boron	1.3	High
Calcium	2940	Very High
Magnesium	425	Very High

Soil analysis was performed by the Soil Testing Laboratory, University of Minnesota. The field was continuously cropped with corn and soybeans in alternate years, starting in the early 1970's. All mineral data listed in PPM, to convert to lbs./acre multiply ppm by 2. Data followed by a (+) were above the threshold value for the processing equipment.

APPENDIX G

HOAGLAND'S NUTRIENT SOLUTION

Appendix G. Hoagland's Nutrient Solution

Prepare at least one day in advance of required use to maximize dissolution of reagents. Prior to all subsequent nutrient dilution preparations visually check stock bottles for microbial contamination. Phosphorus stock is especially susceptible and may remain usable for 4 to 6 weeks. The remaining stock solutions seem to remain free of observable microbial colonies for 6 months.

Weigh out the correct amount of chemical, and add to a volume of water (DD- 6th floor autoclave room) that is less than final volume (300 - 400ml with a 500ml final volume) in a beaker. Stir or swirl the solution until the reagent is completely dissolved. This may take considerable time (e.g., EDTA, $MgSO_4$). Transfer the solution to an appropriate sized volumetric flask and add DD-H_2O to mark (watch meniscus). Add in order listed below.

Stock Compound	**Source**	**FW**	**Dry weight to volume**	**Molar conc.: Stock**	**ml per 10L**	**mg/ml of full strength**	**PPM**
KNO_3	Sigma P6162	101.11	50.55g/500ml	1 M	5	K: 1.955 NO_3: 3.100	1955 3100
$Ca(NO_3)_2$-$4H_2O$	Fisher C-109	236.15	236.15/1L	1 M	5	Ca: 2.004 NO_3: 6.200	2004 6200
$MgSO_4$-$7H_2O$		246.48	123.24g/500ml	1 M	2	Mg: 0.0486 SO_4: 0.192	48.6 192
EDTA-$2.5H_2O$	Sigma-EDFS	412.04	3.9450g/500ml	19.1mM	1		
Micronutrients			x/1L		1	µg/ml	
1x. H_3BO_3	Fisher A-73	61.83	2.86g/L	46mM		BO_3: 2.7	2.7
2x. $MnCl_2$-$4H_2O$	JT Baker 2540	197.91	1.81g/1L	9.1mM		Mn: 0.50 Cl: 0.645	0.50 0.645
3x. $ZnSO_4$-$7H_2O$	JT Baker 4382	287.558	0.22g/L	0.76mM		Zn: 0.0497	0.0497
4x. $CuSO_4$-$5H_2O$	JT Baker 1843	249.68	0.08g/L	0.32mM		Cu: 0.0203	0.0203
5x. $(NH_4)_6Mo_7O_{24}$-$4H_2O$	Sigma M-0878	1236	0.0232g/L (includes purity factor)	0.11mM		Mo: 0.0107	0.0107
KH_2PO_4	Sigma	136.09	68.045g/500ml	1 M	1	K: 0.0391 PO_4: 0.031	39.1 31.0

Mycorrhizal Spore Production Experiment

A 10% Hoagland's nutrient solution was added once weekly. The stock nutrients were added at the level of full strength to one liter and subsequently diluted to a 10L final volume.

45L are needed to water the plants in three growth chambers at the following Liter quantities:

a. Plants with conetainers: 3L (fills tub to base of conetainer where the slope of the tube base changes

b. Plants in pots: 2L

10% Hoagland's in a 10 liter final solution volume yields a pH 5.49.