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# Re-Introduction of Soil Mycorrhizae into Roadside Prairie Planting

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The three objectives of this project were as follows: (1.) to survey the arbuscular mycorrhizal fungi from the prairies, wetlands, and Mn/DOT experimental sites in anticipation of using data as indicators of the sites' health, (2.) to produce mycorrhizal inocula by different methods and (3.) to incorporate the inoculum into a Mn/DOT restoration site. This project was the first year of a three year study that seeks to define the variability in the mycorrhizal spores and other structures present in different ecosystems and determine if these fungi can be used as indicator species.

All phases of the project were successful. The preliminary results suggest the mycorrhizal fungal spore numbers will be useful health indicators of restoration sites. General and single spore mycorrhizal inocula were generated using primarily native grasses and forbs as plant hosts. The general inoculum was then incorporated into a Mn/DOT experimental site. Future evaluations of this site will help to answer long-term questions regarding the possible benefits of amending disturbed roadway plantings or other prairie restorations with mycorrhizal inoculum.

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# RE-INTRODUCTION OF SOIL MYCORRHIZAE INTO ROADSIDE PRAIRIE PLANTING

## Interim Report

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## EXECUTIVE SUMMARY

The establishment of native grasses and forbs along many roadside right-of-ways is one of Minnesota Department of Transportation (Mn/DOT)'s goals. Arbuscular mycorrhizal fungi (AMF) are symbiotic fungi that form mutually beneficial relationships with the roots of many prairie and some wetland plants. AMF serve as biofertilizer by facilitating the uptake of nutrients, especially phosphorus, for the plant; and the plant provides carbohydrates to the fungus. In addition, mycorrhizal plants have greater tolerance to stressful environmental conditions than the non-mycorrhizal plants.

Because of the advantages that AMF confer on many plants, the mycorrhizal condition of plants in newly established prairies is of importance to the plants' survival. In order to restore prairies and wetlands, the below ground soil/organism parameters must be characterized as well as the above ground plants of remnant prairies. At present, little information is available about the plant/mycorrhizal characteristics of remnant and restored prairies in Minnesota.

The project's objectives were threefold: (1) to survey arbuscular mycorrhizal (AM) fungi from the soils collected from prairies, wetlands, and Mn/DOT experimental sites, (2) to produce general and single spore AM inoculum and (3) to incorporate this inoculum into a Mn/DOT restoration site.

The characterization of AMF from several prairies and wetlands reveals a significant difference in AM spore numbers among the prairies of three different ages: remnants, those restored by Mn/DOT in the past 5 years and those restored in the past 10 years. The lowest spore numbers in a gram of dry soil were found at the newly restored prairies and the highest of those restored about 10 years ago. In coming years, if further studies show similar results, AM spore number may be a useful indicator of a prairie's below ground health. However, the results collected in this survey need to be repeated during the next two years to determine the variability in the data.

# CHAPTER 1. INTRODUCTION

The establishment of native grasses and forbs along many roadside right-of-ways is one of Mn/DOT's goals. In such restoration projects, certain characteristics of a site can be analyzed to assess the condition of an ecosystem. Some of these characteristics include the stage of succession, plant composition and the presence of soil symbiotic fungi, such as arbuscular mycorrhizal fungi (AMF). AMF are symbiotic fungi that form mutually beneficial relationships with the roots of many prairie and some wetland plants.

The beneficial effects of AMF in prairie restoration has been summarized by Miller and Jastrow [1]. AMF serve as biofertilizer by facilitating the uptake of nutrients, especially phosphorus, for the plant; and the plant provides carbohydrates to the fungus. In addition, mycorrhizal plants have greater tolerance to stressful environmental conditions such as drought [2] and the presence of high soil heavy metal [3,4] and/or salt concentrations [5,6] than non-mycorrhizal plants.

Because of the advantages that AMF confer on many plants, the mycorrhizal condition of plants in newly established prairies is of importance to the plants' survival. In order to restore prairies and wetlands, the below ground soil/organism parameters must be characterized as well as the above ground plants of remnant prairies. At present, little information is available about the plant/mycorrhizal characteristics of remnant and restored prairies in Minnesota.

The study by Stenlund *et al.* [7] provided the initial characterization of prairie sites and an experimental one amended with grass and forb species. Some of the same sites as well as new ones are characterized in this report. The plant/mycorrhizal parameters that are summarized in this project are being further characterized in the second phase of this study, which is being funded in part by LCMR. Ultimately, Mn/DOT and other state agencies will be provided with (1.) AMF indicator data collected over a three year period and (2.) with the results of experimental sites monitored during their critical formative years, such as ones near Cambridge. The changes that occur in these prairies, wetlands and other natural areas over both short and long periods need to be determined to assure that adequate restoration techniques are used.

Our primary objectives in this project were to:

1. Collect vesicular arbuscular mycorrhizae (VAM) from prairies, sites near the restoration projects and established field experimental plots.
2. Characterize VAM from prairie, sites near restoration projects and existing research plots.
3. Produce VAM inoculum.
4. Install permanent uncontrolled and controlled field experimental plots near Cambridge, MN.
5. Characterize and monitor certain physical and biological parameters of the newly created and undisturbed wetlands near Cambridge, MN.
6. Collect and analyze soil and root samples for VAM determination from an experimental field site selected by Mn/DOT for re-introduction of VAM.
7. Amend grass and forb establishment with VAM.

Chapter 2 is an arbuscular mycorrhizal fungal survey which addresses objectives 1 and 2. Chapter 3 emphasizes objectives 4 and 5, which concern the installation and edaphic, plant and mycorrhizal parameters at three experimental sites: one established at Lake Shetek in 1991 and two others established in 1994 adjacent to a highway and a county road near Cambridge, MN, JES and country club respectively. In general, the time required for a prairie or wetland to be restored to conditions similar to established ones has not been determined. The data will be useful in tracking the development of these sites in future years.

Chapter 4 provides specific information about AMF isolated from a heavy metal contaminated experimental site (objectives 1 and 2). Many road right-of-ways are similarly contaminated with heavy metals; so the ability to produce inoculum of heavy metal tolerant AMF strains for use in roadside native plantings would be beneficial to some restoration projects.

Chapters 5 and 6 deal with AMF inoculum production (see objective 3). Chapter 5 concerns general and single-spore specific inocula generation. The general inoculum was formed in the winter and spring of 1995 for incorporation into the highly disturbed soil at the JES experimental site, Cambridge, MN. The production of a heavy metal tolerant inoculum is detailed in Chapter 6. Objective 6 is not applicable because our Mn/DOT technical liaison did

not select another experimental site for arbuscular mycorrhizae (AM) re-introduction, since the JES site had the characteristics needed for AM amendment. Chapter 7 is about mycorrhizal inoculum field application as outlined in objective 7.

The data summarized in this report increases the limited baseline information available about the biodiversity of AMF in Minnesota prairies and wetlands. Some of the spores and other mycorrhizal structures collected from soils of remnant prairies and undisturbed and disturbed sites were used to generate inocula for spore (species) identification and for re-introduction of AM at a disturbed site. The AM re-introduction done at an experimental site near Cambridge will provide information about the value of using this process at a highly disturbed roadside right-of-way site undergoing restoration.

# CHAPTER 2. MYCORRHIZAL SPORE SURVEY

## 2.1 Overview

One goal of this project is to increase the limited baseline information available about the biodiversity of AM in Minnesota prairies and wetlands [8,9,10]. The variation in the successional patterns in AM communities is the type of information needed to successfully monitor the AM populations at restoration sites from beginning to completion of the projects.

A previous Mn/DOT research project dealt with characterization of AMF from selected prairies in the Twin Cities metropolitan area and in southwestern Minnesota [7]. This part of this study is a continuation of the characterization of AMF from selected prairies and wetlands in Minnesota. Several of the areas studied are the same ones examined by Stenlund *et al.* [7] so that spore data comparisons can be made over a period of years to determine the variability present in the AM fungal population and ultimately to produce AM ecosystem indicator information.

## 2.2 Materials and Methods

### 2.2.1 Survey

Several sites throughout Minnesota were visited during the late summer and fall of 1994. At these sites, the plants were identified in most cases to species (Appendix A), except at three sites, the Cambridge pond SE of JES, Shaefer and Feder Prairies. Soil cores were collected as either a straight line or W transect: transect type/site is listed in Appendix B. After the cores were collected, they were placed in plastic bags which were put on ice in a Styrofoam cooler and transported to the 3°C cold room at the U of MN for storage.

### 2.2.2 Isolation of spores and roots

The cores were broken up and mixed to create a homogeneous composite. After a portion of the soil was removed, the roots were isolated from the soil by running water through a series of soil sieves. The roots on the top sieve were removed with tweezers and the rest of the soil was dried in a convection oven for spore isolation.

The isolated roots were stained immediately, according to the procedure of Kormanik and McGraw [11] or put in 50% ethanol for storage. The soil for spore isolation was placed in cold storage until the spores were isolated according to the procedure given in Appendix C.

## 2.3 Results & Discussion

A number of different wetland and prairie sites were surveyed (Table 2.1) for plant species (Appendix A) and for presence of soil mycorrhizal fungal spores. The age of the three remnant prairies is ~8,000 years according to McAndrews [12]; in contrast, the newly restored ones listed in Table 2.1 range from four to ten years of age. Similar age differences are seen in the remnant wetland versus the newly restored ones. The information about the plant species and spore types is important to provide Mn/DOT and other state agencies with an ecological picture of the individual sites at the beginning of a long term study.

Table 2.1. Survey sites of prairies and wetlands during the summer and fall of 1994.

| Name | Age and characteristics | County* |
|---|---|---|
| **Remnant Prairie** | | |
| •Crosstown Prairie | ~8,000 yrs old | Hennepin |
| •Feder Prairie | ~8,000 yrs old | Blue Earth |
| •Schaefer Prairie (Nature Conservancy) | ~8,000 yrs old | McLeod |
| **Restored Prairie** | | |
| •Lake Shetek | Planted in 1991 | Murray |
| •Materials and Research Laboratory (Mn/DOT) | Planted in 1991 | Ramsey |
| •St. Croix Rest Area (Mn/DOT) | Planted in 1986, burned and unburned parts | Washington |
| •University of Minnesota Horticulture Research Prairie | Planted in mid 1980s | Ramsey |
| **Remnant Wetland** | | |
| •Cambridge-Country Club | Unknown, adjacent to road | Isanti |
| •Schaefer Wetland Nature Conservancy wetland | ~8,000 yrs old, | McLeod |
| **Restored Wetland** | | |
| •Cambridge-JES | Disturbed wetland area | Isanti |
| •Cambridge-Pond SE of JES | Disturbed wetland area | Isanti |
| •Inver Grove Heights-Island Pond | Disturbed wetland area | Dakota |
| •Inver Grove Heights-Undisturb | Undisturbed wetland area | Dakota |

* Locations for these sites may be found in Appendix D.

Because we were concerned about the possible non-homogenous soil/plant conditions which may promote non-random distribution of soil fungal spores especially at the recently restored prairies, we wanted to identify the best transect type to use to collect soil cores for isolations of AMF spores. To test sampling methods, the Mn/DOT Materials and Research Lab Prairie was cored using a straight line transect and then 3 W transects were taken directly adjacent to each other, covering the same length as the straight line. The numbers and colors of AMF spores were compared (Table 2.2). The results obtained using the line transect was compared to a composite of the 3 Ws (Table 2.2). At the Mn/DOT restored prairie the low AMF spore numbers obtained from all transects were statistically the same. Because the line transect is quicker and less expensive to set-up, we used it at the other restored and remnant prairie sites given in Table 2.3.

Table 2.2. Comparison of sampling transects: straight line transect compared to a composite of 3 W transects taken over the same horizontal distance.

| Type of transect | Composite of 3 W Transects | Straight Line Transect |
|---|---|---|
| Spore Color | | |
| Large Brown | 0.7±0.3 | 0.4±0.1 |
| Small Brown | 1.3±0.3 | 1.4±0.4 |
| Large Reddish | 1.5±0.5 | 1.2±0.5 |
| Small Reddish | 0.5±0.1 | 0.6±0.2 |
| Large Yellow | 0.3±0.0 | 0.3±0.1 |
| Small Yellow | 0.6±0.1 | 0.8±0.2 |
| Large Hyaline | 0.5±0.1 | 0.6±0.1 |
| Small Hyaline | 1.0±0.3 | 1.4±0.2 |
| Black | 0.3±0.1 | 0.6±0.2 |
| Green | 0.2±0.1 | 0.2±0.2 |
| Total | $6.2\pm1.0^{a}$ | $7.3\pm1.2^{a}$ |

Data listed are averages ±SE of spore number per gram of dry soil. Identical superscripted letters within a row indicate no significant difference in one-factor ANOVA after log(1+x) transformation unsing the LSD test for mean separation set at the 5% level. (The p-value was below 0.05.)

Characterization of AMF spore number and color per gram of dry soil from remnant and restored prairies are summarized in Table 2.3. There was no difference between the spore number averages at the three remnant prairies (Crosstown, Feder, and Schaefer) and the dominant *Glomus* spore color (brown). The average number of spores at these three sites is 18.3±2.5. However, both of the prairie sites established in the mid 1980s: one by Mn/DOT near the St. Croix River and

the other on the U of MN, St. Paul Campus, have the highest average spore number compared to other prairie types (remnants and more recently restored sites). No statistical difference was found at the St. Croix prairie between the burned and unburned portions; however, monitoring the spore species from the time of the burn until the late fall may be necessary to identify changes in AMF populations [13]. The Mn/DOT Materials and Research Laboratory planted in the early 1990s, had the lowest mean spore number, approximately half the number found at the remnant prairies. The St. Croix and the U of MN restored prairies had the highest spore numbers. This information will be useful for monitoring future prairie restoration sites.

Table 2.3. Arbuscular mycorrhizal spores per gram of dry soil from prairie sites throughout Minnesota. Mn/DOT Materials and Research Laboratory (Mn/DOT Mat & Res), large (l) and small (s)

| | Remnant Prairies | | | | Restored Prairies | | | |
|---|---|---|---|---|---|---|---|---|
| | Crosstown | Crosstown | Feder | Schaefer | Mn/DOT Mat & Res | St. Croix Burned | St. Croix Unburned | U of MN |
| Date Collect → | 10/8 | 11/20 | 11/12 | 10/12 | 10/14 | 10/8 | 10/8 | 10/8 |
| Spore Color ↓ | | | | | | | | |
| l brown | 1.6±0.6 | 3.3±1.6 | 1.1±0.2 | 3.0±0.7 | 0.4±0.1 | 6.9±2.7 | 6.2±1.0 | 9.2±3.5 |
| s brown | 3.8±2.8 | 7.7±1.7 | 13.4±2.5 | 13.2±6.6 | 1.4±0.4 | 8.7±1.9 | 10.2±2.0 | 31.4±6.5 |
| l reddish | 0.6±0.2 | 2.5±1.3 | 0.1±0.1 | 0.3±0.2 | 1.2±0.5 | 1.9±1.1 | 0.1±0.1 | 1.4±0.1 |
| s reddish | 1.1±0.4 | 0.9±0.3 | 0.1±0.1 | 0.1±0.1 | 0.6±0.2 | 11.9±5.5 | 5.3±1.0 | 0 ±0 |
| l yellow | 0.3±0.1 | 0.3±0.1 | 0±0 | 0.2±0.2 | 0.3±0.1 | 1.8±0.7 | 0.6±0.3 | 1.2±0.5 |
| s yellow | 11.3±6.4 | 0.5±0.1 | 0.1±0.1 | 0.1±0.1 | 0.8±0.2 | 2.7±1.5 | 3.1±1.5 | 0.1±0.1 |
| l hyaline | 0.1±0.0 | 0.3±0.2 | 0.5±0.3 | 1.9±0.9 | 0.6±0.1 | 5.1±3.1 | 4.5±2.9 | 1.9±0.5 |
| s hyaline | 0.7±0.7 | 0.7±0.3 | 2.6±0.6 | 3.2±1.4 | 1.4±0.2 | 17.2±6.3 | 14.4±1.9 | 10.5±6.1 |
| black | 0.3±0.2 | 0.3±0.2 | 0±0 | 0±0 | 0.6±0.2 | 3.8±1.6 | 2.9±1.3 | 0±0 |
| green | 0.2±0.1 | 0.0±0.0 | 0±0 | 0±0 | 0.2±0.2 | 0±0 | 0±0 | 0±0 |
| Total | $20.2 \pm 5.7^{a}$ | $16.5 \pm 4.4^{a}$ | $17.8 \pm 2.8^{a}$ | $16.9 \pm 6.9^{a}$ | $7.3 \pm 1.2^{b}$ | $59.9 \pm 11.6^{c}$ | $44.5 \pm 6.2^{c}$ | $43.3 \pm 6.1^{c}$ |

Data listed are averages ±SE of spore number per gram of dry soil. 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. (The p-value was below 0.05.)

Table 2.4 summarizes the number and colors of AMF spores per gram of dry soil from disturbed and undisturbed roadside wetlands as compared to a remnant wetland sedge meadow. In general, a reduction in spore numbers would be expected at the wetter sites. A very low number of

spores was found at the Schaefer sedge meadow and at the JES disturbed roadside wetlands (JES and the pond SE of JES near Cambridge, MN). The high spore number, at the undisturbed roadside wetland site, near the Cambridge Country Club may be the result of the soil/AM gradient extending from a wet to a dry area. The stability of this area is indicated by the high diversity of wetland prairie plants (see Appendix A). The highly disturbed and undisturbed roadside wetlands in Inver Grove Heights were drier than the other sites listed in Table 2.4. Dry soil conditions promote spore formation in the fall and may account for the similar spore numbers obtained. Additional soil samples from other years need to be analyzed to determine the variation in this data.

Table 2.4. Arbuscular mycorrhizal fungal spores per gram of dry soil from wetlands.

| Type of wetland | Remnant wetland (sedge meadow) | Undisturbed Roadside Wetland | Disturbed Roadside Wetlands | | Highly Disturbed Roadside Wetlands | |
|---|---|---|---|---|---|---|
| Name of wetland | Schaefer Wetland | Country Club 10/29 collection | JES 10/29 collection | Pond SE of JES 10/29 | Inver Grove Heights-undisturbed pond | Inver Grove Heights-disturbed pond |
| Spore Color | | | | | | |
| large brown | 0.4±0.2 | 17.5±9.6 | 0.85±0.16 | 0.9±0.1 | 0.7±0.2 | 3.2±2.1 |
| small brown | 2.7±0.4 | 39.7±14.7 | 2.29±0.23 | 3.4±0.2 | 6.3±2.5 | 8.8±1.4 |
| large reddish | 0.3±0.0 | 3.1±1.6 | 0.66±0.2 | 0.2±0.1 | 0.3±0.2 | 1.1±0.4 |
| small reddish | 0.6±0.1 | 2.0±0.7 | 0.1±0.05 | 0.2±0.0 | 0.5±0.3 | 2.9±2.7 |
| large yellow | 0.0±0.0 | 1.7±1.2 | 0.37±0.16 | 0.3±0.1 | 0.6±0.3 | 0.3±0.1 |
| small yellow | 0.2±0.0 | 5.9±5.3 | 0.32±0.18 | 0.2±0.0 | 3.5±2.3 | 1.4±0.6 |
| large hyaline | 0.0±0.0 | 4.7±1.9 | 0.48±0.39 | 0.1±0.1 | 0.3±0.1 | 0.1±0.1 |
| small hyaline | 0.4±0.1 | 3.9±1.1 | 1.08±0.1 | 0.2±0.1 | 1.3±0.6 | 0.5±0.1 |
| black | 0.0±0.0 | 0.0±0.0 | 0.03±0.03 | 0.2±0.0 | 0.3±0.1 | 0.2±0.1 |
| green | 0.1±0.1 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.1±0.1 |
| Total | $4.3\pm0.6^{a}$ | $78.5\pm22.6^{b}$ | $6.17\pm0.65^{ac}$ | $5.8\pm0.3^{ac}$ | $13.8\pm4.6^{cd}$ | $18.5\pm4.3^{d}$ |

Data listed are averages ±SE of spore number per gram of dry soil. 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. (The p-value was below 0.05.)

# CHAPTER 3. EXPERIMENTAL SITES: CHARACTERIZATION OF SOIL AND MYCORRHIZAL STRUCTURES AT NON-HEAVY METAL CONTAMINATED SITES

## 3.1 Overview

AM fungi are present and active in prairies and in wetlands under certain conditions. This chapter deals with the characterization of Mn/DOT experimental sites near Cambridge and Lake Shetek.

The purpose of the Cambridge study was to measure the presence of AM fungal structures and several soil parameters in a wetland recently created in response to the construction of a new highway and in an undisturbed wetland at a later stage of succession, a few miles from the newly created wetland. Many studies indicate that plants growing at disturbed sites in early stages of succession are usually non-mycorrhizal [5]. Then too, during a disturbance, fungal structures can be lost or destroyed. So we predicted that: (1) in the newly created (disturbed) wetland, little, if any, AM fungal spores would be present and (2) in the undisturbed wetland, a relatively large amount of AM fungal spores would be found.

The purpose of the Lake Shetek study was to measure the presence of AM fungal structures and several soil parameters in a wetland prairie in response to mitigation. During the late summer and fall of 1994, five transects (buffer, low density, high density, residual, and undisturbed), established in 1991, were monitored [7]. The low and high density transects were amended with AM fungi. Unfortunately, the area was extensively flooded during the spring and summer of 1993, which has probably effected the data. A comparison of the 1994 data with collections from unflooded years, such as 1995, will help to interpret the results.

## 3.2 Materials and Methods

### 3.2.1 Soil parameters

In July, 1994, the type of transect used to collect soil from the two sites in Cambridge, MN: 1) newly created wetland (JES) and 2) undisturbed wetland (Country Club) is summarized in Appendix B. For the July, 1994 collections, the cores were cut in 1/4's longitudinally in hand-made cardboard template and divided into top 6 cm and bottom 6 cm: A) 1/4 used for

determination of bulk density and percent moisture, B) 1/2 used for remaining tests. The cores were combined into composites of top and bottom from each site. Determination of bulk density, percent moisture and pH were done by the steps outlined in McKeague [14]. Soil samples were sent to Research Analytical Laboratories for determination of nutrient composition. Phosphorus was determined by extractable Bray P-1 method and by the ICP-AES (atomic emission spectrometry with inductively coupled plasma).

For the collections made in September and October, 1994, the cores at the Cambridge site were not separated into top and bottom. The cores collected at the Shetek site at the same time of the year were also not separated. Five cores were taken in each transect and these were composited together. AMF spores were isolated from the soil samples of all the locations listed in Table 2.1 using a method modified from Tommerup and Kidby [15]. The number of AMF spores/g of dried soil and their size and color were determined according to the procedures outlined in Stenlund *et al.* [7].

### 3.2.2 Mycorrhizal percent colonization of roots

The method used for determination of percent colonization is given in Kormanik and McGraw [11].

### 3.2.3. Arbuscular Mycorrhizal Fungal Spore Isolation

Spore Isolation was done by a modified method based on procedures of Tommerup and Kidby [15], (Appendix C).

## 3.3 Results and Discussion

### 3.3.1 Sites near Cambridge, MN

Soil parameters:

The pH of the soil for both parts of the cores taken from the Country Club and JES sites was near neutral (Table 3.4). Because of the similarities between the top and bottom parts of the cores in pH and all other soil parameters, only the results from the bottom part of the cores is given in Table 3.4. The density of soil at the newly created wetland was far greater than the density at the undisturbed site. The percent water (g/cm$^3$) at the undisturbed site was more than twice the

percent water at the newly created wetland. The percent organic matter and total carbon at the undisturbed wetland was greater than at the newly created wetland. More nitrogen in the form of nitrate was recorded at the newly created wetland while more ammonium was found at the undisturbed site. The phosphorus available to plants was greater at the newly created wetland, probably due to phosphorus run off from the surrounding agricultural area. However, as predicted, more total phosphorus was found at the undisturbed site (Table 3.4).

Table 3.4. Comparison of soil parameters at the undisturbed (Country Club) and disturbed (JES) sites near Cambridge, MN (±SE).

| Sites | Country Club (Undisturbed) | JES (Disturbed) |
|---|---|---|
| Soil parameter | | |
| pH | 6.5 | 6.8 |
| Density (g/cm3) | $0.23 \pm 0.02^{a}$ | $1.92 \pm 0.07^{b}$ |
| % Water (g/cm3) | $88.08 \pm 6.00^{a}$ | $40.22 \pm 2.32^{b}$ |
| % Organic Matter | 40.3 | 1.3 |
| Carbon | 20.4 | 0.8 |
| Nitrate (N03-) | <0.5 | 1.8 |
| Ammonium (NH4) | 10.7 | 0.9 |
| Phosphorus (P) {Bray Extractable} | 9 | 15 |
| P (ICP) | 2226.45 | 254.35 |

Data listed are averages ±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. (The p-value was below 0.05.)

Percent root colonization:

No colonization was found in the few roots isolated from the bottom parts of the JES site cores; however, 3.8±1.5 percent colonization was recorded from the Country Club site cores. Similar results were obtained from the top parts of the cores. This significant difference in AM root colonization was anticipated given the scarcity of vegetation at the disturbed JES site as compared with plant density at the undisturbed Country Club location (refer to plant lists in Appendix A).

AMF spore characterization:

At all collection dates, a significantly larger number of AMF spores per gram of dry soil was found at the undisturbed wetland (Country Club) than at the newly created wetland (JES) and

the pond SE of it (Table 3.5). A drop in spore number was observed at the undisturbed site from July to October, which may be a reflection of the increase in water level that occurred during the fall. Little change in spore number was recorded from summer through the fall at the JES disturbed site as expected due to the presence of little vegetation.

Table 3.5. Arbuscular mycorrhizal spores per gram of dry soil collected at the JES disturbed and the Country Club undisturbed sites in Cambridge, MN at summer and fall, 1995 (July 2, Sept. 21, Oct. 29, and May 14).

| Spore color | JES | Country Club | JES | Country Club | JES | Country Club | Pond SE of JES |
|---|---|---|---|---|---|---|---|
| Date | 7/2/94 | 7/2/94 | 9/21/94 | 9/21/94 | 10/29/94 | 10/29/94 | 10/29/94 |
| l brown | 2.0±0.3 | 20.6±2.9 | 0.4±0.1 | 31.0±9.7 | 0.9±0.2 | 17.5±9.6 | 0.9±0.1 |
| s brown | 0.9±0.2 | 16.7±2.2 | 2.6±1.0 | 24.9±2.1 | 2.3±0.2 | 39.7±14.7 | 3.4±0.2 |
| l reddish | 0.1±0.06 | 13.1±2.2 | 0.5±0.2 | 15.0±4.0 | 0.7±0.2 | 3.1±1.6 | 0.2±0.1 |
| s reddish | 0.1±0.03 | 3.6±0.8 | 0.2±0.0 | 1.5±0.3 | 0.1±0.1 | 2.0±0.7 | 0.2±0.0 |
| l yellow | 3.2±0.8 | 13.4±5.0 | 0.1±0.1 | 8.4±3.0 | 0.4±0.2 | 1.7±1.2 | 0.3±0.1 |
| s yellow | 3.9±0.6 | 82.1±9.1 | 0.4±0.2 | 9.7±3.7 | 0.3±0.2 | 5.9±5.3 | 0.2±0.0 |
| l hyaline | 2.9±0.3 | 7.3±1.6 | 0.1±0.0 | 7.8±2.8 | 0.5±0.4 | 4.7±1.9 | 0.1±0.1 |
| s hyaline | 10.3±1.7 | 70.4±4.4 | 1.0±0.2 | 15.0±5.5 | 1.1±0.1 | 3.9±1.1 | 0.2±0.1 |
| Black | 0.9±0.1 | 26.8±2.4 | 0.0±0.0 | 0.1±0.1 | 0.0±0.0 | 0.0±0.0 | 0.2±0.0 |
| Green | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| Total | $24.2\pm3.4^{ad}$ | $253.9\pm23.4^{b}$ | $5.3\pm1.7^{c}$ | $147.0\pm47.0^{d}$ | $6.2\pm0.7^{c}$ | $78.5\pm22.6^{d}$ | $5.8\pm0.3^{c}$ |

Data listed are averages ±SE of spore numbers per gram of dry soil. 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. (The p-value was below 0.05.)

A high level of spore diversity, according to color and size which is species specific, was found at both the undisturbed and disturbed wetland sites. In the earliest collections (7/2/94), the small hyaline and small yellow spores were most frequently recorded at both sites. The high diversity at the disturbed site was unexpected, but may be due to the original wetland soil stored and replaced on this area during restoration.

### 3.3.2 Lake Shetek site

Soil parameters:

Soil cores were taken from the uncontrolled and controlled field experimental plots established in 1991 when AM was re-introduced at the Lake Shetek mitigation site [7]. The soil mineral analysis is given in Stenlund *et al.* [7].

Percent root colonization:

The AMF percent colonization inside the roots of the plant cover was calculated (Table 3.6). No variations in colonization was observed in the September collections. No differences were observed among the buffer, high density and residual transects; however, the percent colonization was significantly lower in the undisturbed transect as compared to the low density.

Table 3.6. Percent colonization of samples collected from Lake Shetek on 10/15/94 (±SE).

| % arbuscular mycorrhizal colonization* | | | | | |
|---|---|---|---|---|---|
| Date | Buffer | Low Density | High Density | Residual | Undisturbed |
| 9/9/94 | 9.3±3.4[a] | 9.8±1.3[a] | 7.3±1.3[a] | 15.3±1.3[a] | 9.3±0.9[a] |
| 10/15/94 | 11.6±1.7[ab] | 24.1±2.2[a] | 18.2[ab] | 21.2[ab] | 10.1±2.8[b] |

Data listed are averages ±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. (The p-value was below 0.05.)

*Consists of internal mycorrhizal structures, i.e.. vesicles

AMF spore characterization:

To evaluate the presence of the AM re-introduced in 1991 at the Lake Shetek wetland mitigation/prairie restoration site [7], we analyzed a AMF spore sample from each of the transects (see Table 3.7). Little spore variation across the transects was observed by Stenlund *et al.* [7], and the same is the case for spore number collected from the buffer, high density and undisturbed transects at Lake Shetek site in October, 1995. The original AMF spore numbers for each of the transects were higher in 1991 [7] than in 1994 (Table 3.7) . We hypothesized that the higher original spore number was due to the prior agricultural use of the field [9]. Now that the Lake Shetek area has been reestablished as prairie, lower values would be expected as indicated by other prairie studies [16].

Table 3.7. Arbuscular mycorrhizal spores per gram of dry soil from five transects at the Lake Shetek site. Collected on 10/15/94 (±SE).

| Spore Color | Buffer Transect | Low Density Transect | High Density Transect | Residual Transect | Undisturbed Transect |
|---|---|---|---|---|---|
| Large Brown | 1.0±0.6 | 1.4±0.1 | 2.4±1.4 | 3.5±1.5 | 3.6±0.5 |
| Small Brown | 15.6±3.8 | 15.7±1.3 | 28.8±21.1 | 33.7±13.5 | 31.7±3.9 |
| Large Reddish | 0.3±0.1 | 0.1±0.0 | 0.9±0.8 | 0.2±0.1 | 0.6±0.3 |
| Small Reddish | 0.3±0.3 | 0.2±0.2 | 1.0±0.5 | 0.2±0.3 | 0.2±0.2 |
| Large Yellow | 0.3±0.2 | 0.2±0.1 | 0.2±0.2 | 0.3±0.2 | 2.6±2.1 |
| Small Yellow | 0.3±0.2 | 1.0±0.2 | 0.4±0.3 | 0.3±0.1 | 0.4±0.3 |
| Large Hyaline | 1.0±0.5 | 0.6±0.0 | 1.3±0.7 | 0.6±0.3 | 5.1±2.3 |
| Small Hyaline | 2.8±0.7 | 5.4±1.4 | 4.0±1.3 | 5.2±2.0 | 6.8±1.7 |
| Black | 0.0±0.0 | 0.0±0.0 | 0.2±0.3 | 0.0±0.0 | 0.0±0.0 |
| Green | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| Total | $23.9\pm3.6^{ab}$ | $18.7\pm1.7^{a}$ | $34.0\pm12.1^{ab}$ | $38.2\pm4.6^{b}$ | $39.0\pm5.1^{ab}$ |

Data listed are averages ±SE of spore numbers per gram of dry soil. 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. (The p-value was above 0.05.)

The biological and physical tests completed in this study will be used on the experimental sites during progressive stages of succession to compare to these preliminary results at these sites. Further monitoring of the site is needed to determine if these trends continue.

# CHAPTER 4. ARBUSCULAR MYCORRHIZA IN PLANT INTERACTIONS WITH HEAVY METALS - A survey of AM fungi at the heavy metal contaminated site: Pig's Eye landfill

## 4.1 Overview

Despite a marked decrease in deposition of lead (Pb), cadmium (Cd) and zinc (Zn) observed in the last decades since introduction of unleaded gasoline in about 1970, trace elements already accumulated in the terrestrial environment due to anthropogenic emissions pose a serious problem [17]. Metals are immobilized in soil by binding to soil colloidal components. They can, however, be easily mobilized due to changes of soil chemical properties such as a decrease in pH, change in the redox potential or enhanced decomposition of organic matter [18]. Cycling and retention of metals in soils do not depend only on physio-chemical soil properties. Both phenomena can be strongly modified by vegetation associated with a particular soil type. Zinc and copper (Cu), which are essential plant micronutrients are readily incorporated and cycled in vegetation; whereas lead, which is not an essential element for plants, is retained and accumulates in the soil [19]. Numerous studies have shown that vegetation in the vicinity of roads with heavy motor vehicular traffic may have enhanced concentrations of heavy metals, which can contribute to the introduction of these elements into food chains [20].

Arbuscular mycorrhiza (AM), a mutualistic association between AM fungi (Glomales, Zygomycetes) and the roots of most land plants [21] appears to play an important role in plant interactions with trace metals. The AM fungi are obligate symbionts obtaining carbohydrates from a host plant and contributing to host plant improved acquisition of mineral nutrients, such as phosphorus, copper and zinc when these are present in soil at low concentrations [22]. The protective function of mycorrhizal symbiosis against heavy metal stress has been well documented in ericoid mycorrhiza [23] as well as in certain ectomycorrhizae [24]. It still, however, remains largely unclear in case of AM fungi. Tolerance to extreme heavy metal exposure has been reported in several species of AM fungi [25]. The AMF were shown to protect a host plant from excessive

uptake of some heavy metals [26]. On the other hand, AM enhances absorption of metals even if they occur in excess, which may have a detrimental effect on a host plant [27].

Mechanisms of heavy metal metabolism in AMF are largely unknown. Fungal cells can regulate intracellular levels of potentially toxic metals by limiting concentrations of free metal ions via extracellular precipitation and complexation, binding to cell walls, sequestration by metal binding proteins and polypeptides and vacuolar compartmentation often in the form of polyphosphate granules [28].

In order to investigate the role of AMF in plant interactions with heavy metals, soil and root samples were collected from experimental plots established by M. Chin and R. L. Chaney at the Pig's Eye landfill, St. Paul, MN, which is contaminated with Cd, Cu and Pb. AMF spore occurrence was assessed in the rhizospheres of selected plant species. Arbuscular mycorrhizal colonization was quantified in corn roots.

## 4.2 Materials and Methods

### 4.2.1 Site description

Soil at the Pig's Eye landfill, St. Paul, MN, is characterized by the enhanced concentrations of Cd, Cu and Pb resulting from a long term disposal of concentrated municipal wastes [29].

In the autumn of 1993, samples of soil and roots of selected plant species (red fescue, *Festuca rubra* L., campion, *Silene cucubalus* Wibel-Fernald, penny-cress, *Thlaspi caerulescens* J. & C. Presl., corn, *Zea mays* L.), introduced on experimental plots established by M. Chin and R. L. Chaney in 1991, were collected. Prior to plant introduction, the experimental plots were treated with sulfur and two forms of nitrogen fertilizer: either calcium nitrate or ammonium sulfate in order to obtain a soil environment of varying pH levels.

### 4.2.2. Sampling

When possible, soil associated with roots of the 4 specimens or clusters of specimens from each soil treatment was collected. Corn root balls containing clustered roots of 3-4 plants were subdivided into two parts: one was dried and used for AMF spore isolation (similarly as in

remaining species) as well as for soil chemical analyses, the other part was used as a source of fresh corn roots for quantifying AM colonization.

#### 4.2.3. Soil properties

Preliminary analyses of heavy metal concentrations in three randomly chosen soil samples were performed by ICP (atomic emission spectrometry with inductively coupled plasma) after 1N $HNO_3$ microwave digestion. The pH of soil associated with the corn root systems (root balls) was measured in water and $CaCl_2$. Soil treated with a nitrogen source and sulfur had a neutral pH; while soil treated with only a nitrogen source had a higher pH.

#### 4.2.4. Mycorrhizal spore analysis

The AMF spores were isolated from soil associated with root balls by wet sieving and decanting followed by sucrose centrifugation (Appendix C). Total numbers of AMF spores found in soil from plant root balls were assessed using a dissecting microscope. Spore voucher slides were deposited in Dr. Charvat's lab.

#### 4.2.5. Corn root AM colonization

Roots were rinsed in tap water and fixed in ethanol for storage [30]. After clearing in hot 10% KOH (w/v), roots were acidified with 1% HCl and stained in 0.1% chlorazol black E [31]. Percent root colonization was assessed by a magnified intersection method in two subsamples of each corn root system and were averaged afterwards [32].

### 4.3 Results and Discussion

#### 4.3.1. Soil properties

Preliminary analyses of heavy metal content in soil from the landfill site indicated that concentrations of heavy metals are higher than in the uncontaminated soils (Table 4.8).

Table 4.8. Comparison of heavy metal concentrations in Pig's Eye soil and worldwide mean values for uncontaminated soils (mg kg $^{-1}$) according to Kabata-Pendias and Pendias [33]. Values are means ± 1 SE.

| Element | Metal concentration in landfill soil in mg kg$^{-1}$ | Worldwide mean values |
|---|---|---|
| Cd | 35 ± 10 | 0.5 |
| Cr | 150 ± 25 | 54 |
| Cu | 160 ± 30 | 18 |
| Mn | 450 ± 15 | 440 |
| Ni | 45 ± 10 | 22 |
| Pb | 160 ± 25 | 32 |
| Zn | 330 ± 70 | 71 |

### 4.3.2. Mycorrhizal spore analysis

The range of AMF spore numbers for all analyzed samples was from 3 to 61 spores per gram of dry soil. Lack of significant differences in the spore numbers depending on the species of a source plant was found. Even though, *T. caerulescens* and *S. cucubalus* are considered not to be AM host species, growth of ephemeral weedy plants in their vicinity may have resulted in the increase of spore counts [34]. A slightly higher average numbers of spores associated with corn roots may reflect a AM host nature of this species. Examination of root material recovered during spore isolation procedure indicated that on average higher numbers of spores were associated with corn root debris in comparison to *T: caerulescens* and *S. cucubalus* root debris.

### 4.3.3. Percent colonization in corn roots

Analysis of AM colonization of corn roots revealed presence of arbuscules, indicating a functional AM association, as well as vesicles, considered to be storage structures of AM fungi. The highest average arbuscular mycorrhizal (AM) colonization was found in roots of corn plants grown on plots amended with a nitrogen source and sulfur. The lowest average AM colonization had roots of plants grown on plots fertilized with only a nitrogen source.

Presence of arbuscular colonization in the analyzed corn roots indicated that the symbiotic relationship developed at the heavy metal contaminated site was functional [35]. The pH lowering effect of sulfur, seemed to promote development of AM colonization. Ammonium sulfate

suppresses AM development in corn roots. Similar results were obtained in *Chrysanthemum morifolium* grown in hydroponic sand cultures where nitrate nitrogen stimulated, whereas ammonium source of nitrogen suppressed mycorrhizal colonization [36]. The role of AM fungi in mediating nitrogen nutrition of host plants is relatively less investigated than their profound role in phosphorus nutrition. It has been demonstrated, however, that AM fungi differ in their preferences for the nitrogen source and depending on the preferred source of nitrogen may improve plant acquisition of nitrate or ammonium [37]. To estimate the optimal soil edaphic conditions for AM fungi at restoration sites such as JES, experimental plots treated with various soil amendments would be valuable.

Occurrence of AMF spores in soil and AMF colonization in roots of plants introduced to the heavy metal contaminated site indicates a potential role of AM in plant interaction with heavy metals. Based on the results of the effect of nitrogen source on the extent of colonization of corn roots, AM symbiosis may be not only involved in plant interaction with heavy metals but also affect overall plant mineral nutrition under a heavy metal stress. A potential role of AM fungi in mediation of plant heavy metal uptake indicates that soil and plant heavy metal content should be assessed at restoration sites before establishment and after completion of restoration projects.

# CHAPTER 5. INOCULUM PRODUCTION

## 5.1 Overview

Establishment of efficient methods for the production of AM inoculum of known origin and defined characteristics is important for use in restoration projects, such as along highways. Unfortunately, mycorrhizal inoculum production is frequently expensive and time consuming because AMF must be cultured in living roots. Stenlund *et al.* [7] successfully produced a mycorrhizal general inoculum for use at a Mn/DOT mitigation site. As was the case in that study [7], in this investigation native prairie host plants were inoculated with AMF and were grown in the greenhouse and growth chambers in the fall and winter to generate inoculum for the late spring restoration planting. However, in this inoculum production the following plants were used: an agricultural plant, *Zea mays* L.(corn), known to generate high numbers of AM spores [38] and four additional native plants including two legumes.

Production techniques were established in the greenhouse and growth chambers to test methods of AMF production using different native species and environmental conditions. The goals of the inoculum production were to (1.) increase the number of AMF structures available in the general inoculum for use at Mn/DOT restoration project and (2.) to use the single spore inoculum pots for isolation for spore identification of site specific species.

## 5.2 Materials and Methods

### 5.2.1 General inoculum

Four different plant species, little bluestem (*Schizachyrium scoparium* (Michx.) Nash); side-oats grama (*Bouteloua curtipendula* (Michx.) Torr.); purple prairie clover (*Petalostemum purpureum* (Vent.) Rydb.) inoculated with *Rhizobium*, and leadplant (*Amorpha canescens* Pursh.) were germinated in flats. A 2 cm layer of sterile greenhouse soil (from the CBS greenhouse at the U of MN, covered by a 1 cm layer of a 1:1 mixture of coarse vermiculite (Koos Inc., Kanosha, WI) and coarse perlite ( Midwest Perlite Co. Appleton, WI) was placed in each flat. The seeds were spread on top of this layer and then covered by another 2 cm layer of perlite:vermiculite mix.

After germination, the seedlings were inoculated with soil collected from Crosstown Prairie on Nov. 20, 1994. This soil was dried in the convection oven and then turned into a fine grain by grating it on a 2 mm metal sieve. This fine soil was then placed in a round 10 cm diameter pot between two layers of CBS greenhouse steam sterilized soil ( 730 $cm^3$ of sterilized soil on the bottom, 100 $cm^3$ of inoculum soil with 340 $cm^3$ of sterilized soil on top).

The soil in the pots was moistened prior to planting by placing the pots in flats filled with distilled water and allowing the water to diffuse into them. Three seedlings of all four of the plant species were placed in every pot, each in a 2 1/2 cm deep hole (Figure 5.1). Twenty pots contained the 1 $cm^3$ layer of inoculum soil, and five control pots contained a layer of sterile soil instead of the inoculum layer. After planting, the pots were left in the flats in the lab room overnight to absorb more water, and then were placed in the growth chamber or in the greenhouse, depending on the size and health of the shoot. Pots planted with little bluestem were placed in a growth chamber for three weeks and then moved to the greenhouse when the plants were large enough. *Rhizobium* nodules were present on 71 out of 75 of the purple prairie clover seedlings.

The plants were watered with 10% Hoagland's (see Appendix E [39]) once a week and distilled water as needed. They were grown through flowering and the beginning of senescence, about 16 weeks. After this time, watering was stopped, and the plants were allowed to dry out. Before this drying process occurred, cores were taken to preserve some roots. The roots were isolated from the soil and placed in 50% ethanol, according to the procedure outlined in Appendix F. After at least 30 days, the shoot was removed and placed in a paper bag, which was dried in the oven. The roots and the soil were then placed in plastic bags and stored in the cold room until used for AM re-introduction experiment at the JES site in Cambridge, MN (see Chapter 7).

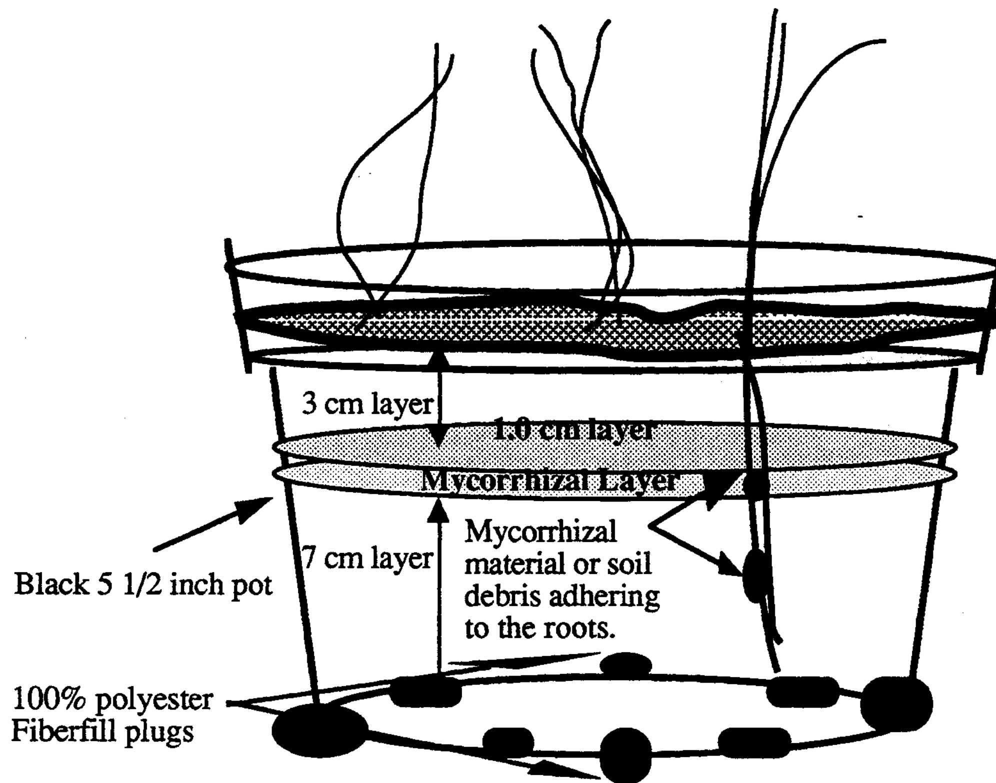


Figure 5.1. General inoculum set-up: Three little bluestem (*Schizachrium scoparium*), side-oats grama (*Bouteloua curtipendula*), purple prairie clover (*Petalostemum purpureum*), or leadplant (*Amorpha canescens*) plants were placed in each pot along with a layer of inoculum soil.

### 5.2.2 Single spore inoculum produced from prairie isolate spore

Four different plant species, big bluestem (*Andropogon gerardii* Vitman), side-oats grama, (*Bouteloua curtipendula* (Michx.) Torr.), corn (*Zea mays* L.) and blue joint grass (*Calamagrostis canadensis* (Michx.) P. Beauv.), were germinated in flats. All the seeds, except the corn *(Zea mays)*, were planted in a 2 cm layer of greenhouse soil (from the CBS greenhouse, U of MN), followed by a 1 cm layer of a 1:1 mixture of coarse vermiculite (Koos Inc., Kanosha,WI) and coarse perlite (Midwest Perlite Co. Appleton, WI), seeds, and another 2 cm layer of perlite:vermiculite mix. The corn *(Zea mays )* seeds were surface sterilized and then germinated on moist paper towels in a dark growth chamber.

After germination, the seedlings of big bluestem, side-oats grama, and corn were inoculated with spores isolated, according to the procedure for single spore inoculum found in Appendix C, from soil collected from Crosstown Prairie on Oct. 8, 1994. Calgon was used to isolate some of the spores that inoculated corn and side-oats grama. This soil was dried in the convection oven and then placed in the cold room for a cold treatment of 2 to 4 weeks before spore isolation. While the seedlings of blue joint grass were inoculated with spores isolated (see Appendix C) from either the Country Club or JES sites at Cambridge, MN. In addition, some of the spores were isolated from soil collected in July, 1994 and stored in the cold room, because of a lack of certain spore types.

One plant was placed in each round 10 cm diameter pot (see Figure 5.2). The soil in the pots was moistened prior to planting by filling flats with distilled water and allowing the water to diffuse into them. Each seedling was placed in a 2 1/2 cm deep hole. After planting, the pots were put in the growth chamber.

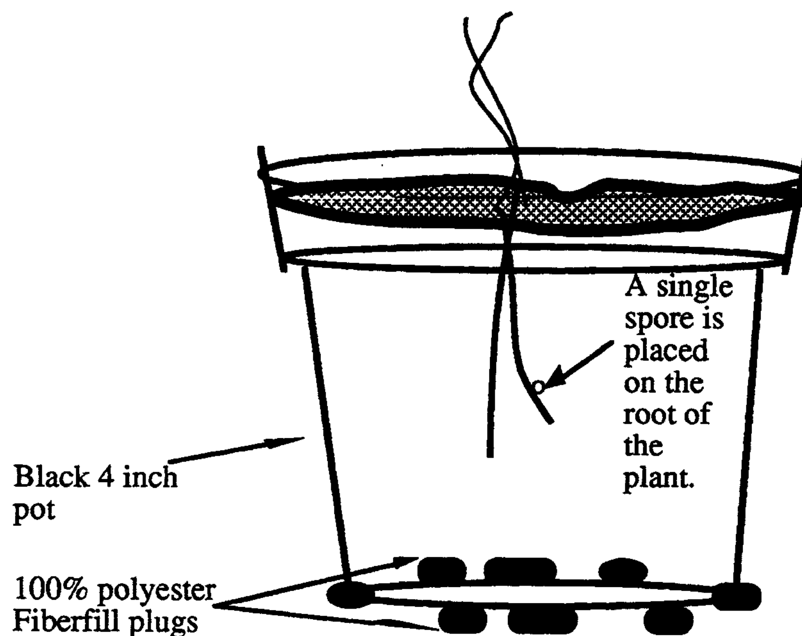


Figure 5.2. Single spore inoculum set-up: One big bluestem *(Andropogon gerardii)*, side-oats grama *(Bouteloua curtipendula)*, or blue joint grass *(Calamagrostis canadensis)* plant was placed in each pot and inoculated with a single spore.

The plants were given 10% Hoagland's solution, once a week, and distilled water as needed. Filter paper was placed on the surface of each for the last half of the experiment to try to prevent contamination from other spore sources. They were grown through flowering and the beginning of senescence, about 16 weeks. After this time, they were no longer watered and were allowed to dry out. Before they were dried out, however, cores were taken to preserve some roots. Processing of the roots and shoots were identical to the methods described in 5.2.1.

### 5.2.3. Single spore inoculum produced from wetland isolated spore

Blue joint grass, *Calamagrostis canadensis*, was germinated in flats. The seeds were planted in a 2 cm layer of University of Minnesota CBS greenhouse soil mix, followed by a 1 cm layer of a 1:1 mixture of coarse vermiculite (Koos Inc., Kanosha,WI) and coarse perlite ( Midwest Perlite Co. Appleton, WI), seeds, and another 2 cm layer of perlite:vermiculite mix.

After germination, the seedlings were inoculated with spores isolated, according to the procedure for single spore inoculum found in Appendix C, from soil collected from the JES or the Country Club sites at Cambridge, MN. Some of the spores were isolated from soil collected in July, 1994 and stored in the cold room, because of a lack of certain spore types. This soil was dried in the convection oven and then placed in the cold room for a cold treatment before spore isolation occurred.

The inoculated blue joint grass was planted using the same procedures as for the prairie plants in 5.2.2, except that the soil was steam sterilized for two hours, permitted to cool for at least 24 hours, and then steamed again. The plants were transferred to the greenhouse and watered with distilled water as needed and once a week with 10% Hoagland's with phosphorous for the first three weeks and then with 10% Hoagland's solution without phosphorous for the rest of the growing period. The soil was kept moist by placing the pots in containers filled with distilled water. The plants were grown for about 16 weeks. Plant and soil drying, root isolation and fixation procedures are the same as for the prairie plants (5.2.2).

### 5.3 Results and Discussion

In general, the plants selected as AMF hosts, germinated and withstood transplant stress well (Table 5.9). The exceptions were as follows: blue joint grass, which showed signs of transplant stress and watering difficulties; lead plant, a perennial, which is known to be difficult to germinate and grows slowly; and side-oats grama, which was too young to transplant at two weeks. Because of this transplant stress, four week old side-oats grama seedlings were used as hosts for the general inoculum; all of these plants survived (see Table 5.9).

The leadplant, a shrub species, is still growing and will be checked for AMF spores when it flowers or by next fall.

Table 5.9. Plant species data, growth conditions, and spore sources for production of mycorrhizal inocula.

| Species | # of Plants | Growing Conditions | Type of Inoculation | Spore Source | # of plants survived | Reasons for deaths |
|---|---|---|---|---|---|---|
| leadplant | 20 | Upland | General Inoculum | Crosstown Prairie | 12 | Transplant stress |
| side-oats grama | 75 | Upland | General Inoculum | Crosstown Prairie | 75 | All survived |
| purple prairie clover | 75 | Upland | General Inoculum | Crosstown Prairie | 75 | All survived |
| little bluestem | 75 | Upland | General Inoculum | Crosstown Prairie | 75 | All survived |
| big bluestem | 80 | Upland | Single Spore | Crosstown Prairie | 80 | All survived |
| side-oats grama | 72 | Upland | Single Spore | Crosstown Prairie | 48 | Seedlings were too young, transplant stress |
| blue joint grass | 50 | Upland | Single Spore | JES Pond | 42 | Transplant stress and difficulty in watering |
| blue joint grass | 60 | Upland | Single Spore | Undisturbed Cambridge Wetland | 41 | Transplant stress and difficulty in watering |
| blue joint grass | 28 | Wetland | Single Spore | Undisturbed Cambridge Wetland | 28 | All survived |
| corn | 80 | Upland | Single Spore | Crosstown Prairie | 80 | All survived |

We wanted to improve AMF culturing methods so that large amounts of inocula can be produced commercial. The achievement of a high reproduction rate of AM fungi in pot cultures is one of the most essential steps in a successful restoration process. The choice of the proper culture parameters such as host plant species, concentration of nutrients, watering, which promote extensive root colonization and/or spore formation, is of great importance. The more traditional general inoculum production method discussed in this chapter required a four month period to generate the AM in the roots of native plant for re-inoculation at the Mn/DOT JES site (see Chapter 7). The most appropriate field samples collected as part of objective 1 (see Chapter 2) served as the original inocula to generate more AMF for the JES, Cambridge restoration project. Very high reproduction of a few spores (large and small yellow and small hyaline) were recorded (Table 5.10). Most of the spore types increased moderately; however, a few showed slight or no reproduction in soil pot cultures watered manually.

In contrast to the soil/sand general inoculum generated by Stenlund *et al.* [7], only greenhouse soil was used as the pot substrate to determine if more spores would be produced in soil only and if less watering would be required. Although many spores reproduced at a reasonable rate (Table 5.10), the sand/soil mixture generated more reproducible propagules [7], so this substrate will be used in our future experiments.

Table 5.10. Spore number per gram in greenhouse pots inoculated with general inoculum. Final inoculum concentration reflects spore numbers only. (Abb: CTP = Crosstown prairie, LB = Little bluestem *(Schizachyrium scoparium)* and SOG = Side-oats grama *(Bouteloua curtipendula)*.

| Inoculum Source | Host Plant | Spore Type | Spore Number /g Initial | Spore Number /g Final | Production (x fold) Increase | Frequency Initial (%) | Frequency Final (%) | Frequency Percent Change |
|---|---|---|---|---|---|---|---|---|
| CTP | LB | Large Brown | 0.312 | 0.5 | 1.6 | 20.0 | 1.3 | -93.5 |
| | LB | Small Brown | 0.729 | 9.4 | 12.9 | 46.7 | 24.9 | -46.7 |
| | LB | Large reddish | 0.237 | 0.5 | 2.1 | 15.2 | 1.3 | -91.4 |
| | LB | Small reddish | 0.085 | 0.8 | 9.4 | 5.4 | 2.0 | -63.0 |
| | LB | Large Yellow | 0.028 | 18.8 | 671.4 | 1.8 | 49.4 | 2644.4 |
| | LB | Small Yellow | 0.047 | 7.7 | 163.8 | 3.0 | 20.3 | 85.2 |
| | LB | Large Hyaline | 0.028 | 1.3 | 46.4 | 1.8 | 3.5 | 93.3 |
| | LB | Small Hyaline | 0.066 | 17.3 | 262.1 | 4.2 | 45.6 | 985.7 |
| | LB | Black | 0.028 | 0.2 | 7.1 | 1.8 | 1.8 | 0.0 |
| | LB | Green | 0.003 | 0.1 | 33.3 | 0.2 | 1.0 | 400.0 |
| | LB | Total | 1.562 | 38.0 | 24.3 | 100.0 | 100.0 | 0.0 |
| CTP | SOG | Large Brown | 0.312 | 0.1 | 0.3 | 20.0 | 2.8 | -86.0 |
| | SOG | Small Brown | 0.729 | 2.3 | 3.2 | 46.7 | 44.6 | -4.5 |
| | SOG | Large reddish | 0.237 | 0.2 | 0.8 | 15.2 | 3.0 | -80.3 |
| | SOG | Small reddish | 0.085 | 0.4 | 4.7 | 5.4 | 7.9 | 46.3 |
| | SOG | Large Yellow | 0.028 | 0.8 | 28.6 | 1.8 | 16.3 | 805.6 |
| | SOG | Small Yellow | 0.047 | 1.2 | 25.5 | 3.0 | 22.8 | 660.0 |
| | SOG | Large Hyaline | 0.028 | 0.7 | 25.0 | 1.8 | 13.4 | 644.4 |
| | SOG | Small Hyaline | 0.066 | 3.6 | 54.5 | 4.2 | 71.1 | 1592.9 |
| | SOG | Black | 0.028 | 0.1 | 3.6 | 1.8 | 0.6 | -66.7 |
| | SOG | Green | 0.003 | 0.1 | 33.3 | 0.2 | 0.2 | 5.0 |
| | SOG | Total | 1.562 | 5.1 | 3.3 | 100.0 | 100.0 | 0.0 |

The production of the single spore inocula gave highly variable results as anticipated, because the probability of one spore colonizing a root system is low (Table 5.11). However, we needed to do single spore inoculum to help generate populations for AMF spore identification. We are working on the further characterization of these spores to species, where possible. Single spore inocula, most likely, would not be pursued by commercial interests due to its limited applications.

Table 5.11. Spore number per gram in greenhouse pots inoculated with single spore inoculum. Final inoculum concentration reflects spore numbers only. (Country Club (CC) and the disturbed JES wetland (JES))

| Inoculum | Spore | Spore Number /g | | | Production | | Frequency |
|---|---|---|---|---|---|---|---|
| Source | Type | Initial | Final | (x fold) Increase | Initial (%) | Final (%) | Percent Change |
| CC* | Large Brown | 0.002 | 0.3 | 130.0 | 10 | 11.3 | 12.7 |
| | Small Brown | 0.002 | 1.7 | 845.0 | 10 | 73.9 | 639.4 |
| | Large reddish | 0.002 | 0.1 | 65.0 | 10 | 5.5 | -45.1 |
| | Small reddish | 0.002 | 0.0 | – | 10 | 0.0 | -100.0 |
| | Large Yellow | 0.002 | 0.0 | 10.0 | 10 | 0.9 | -91.2 |
| | Small Yellow | 0.002 | 0.0 | 10.0 | 10 | 0.9 | -91.2 |
| | Large Hyaline | 0.002 | 0.0 | – | 10 | 0.0 | -100.0 |
| | Small Hyaline | 0.002 | 0.1 | 65.0 | 10 | 5.8 | -42.3 |
| | Black | 0.002 | 0.0 | – | 10 | 0.0 | -100.0 |
| | Green | 0.002 | 0.0 | 20.0 | 10 | 1.3 | -87.0 |
| | Total | 0.02 | 2.3 | 114.0 | 100 | 100.0 | 0.0 |
| CC** | Large Brown | 0.002 | 0.2 | 115.0 | 10 | 11.5 | 14.5 |
| | Small Brown | 0.002 | 1.2 | 615.0 | 10 | 61.8 | 518.3 |
| | Large reddish | 0.002 | 0.3 | 130.0 | 10 | 12.8 | 28.2 |
| | Small reddish | 0.002 | 0.1 | 40.0 | 10 | 4.1 | -59.1 |
| | Large Yellow | 0.002 | 0.1 | 65.0 | 10 | 6.8 | -32.5 |
| | Small Yellow | 0.002 | 0.0 | 20.0 | 10 | 2.0 | -79.6 |
| | Large Hyaline | 0.002 | 0.1 | 35.0 | 10 | 3.7 | -63.0 |
| | Small Hyaline | 0.002 | 0.2 | 85.0 | 10 | 8.3 | -17.0 |
| | Black | 0.002 | 0.0 | 0.0 | 10 | 0.0 | -100.0 |
| | Green | 0.002 | 0.0 | 0.0 | 10 | 0.0 | -100.0 |
| | Total | 0.02 | 2.0 | 100.0 | 100 | 100.0 | 0.0 |
| JES* | Large Brown | 0.002 | 0.8 | 410.0 | 10 | 19.7 | 97.2 |
| | Small Brown | 0.002 | 2.3 | 1165.0 | 10 | 55.8 | 458.3 |
| | Large reddish | 0.002 | 0.1 | 70.0 | 10 | 3.4 | -66.4 |
| | Small reddish | 0.002 | 0.1 | 55.0 | 10 | 2.6 | -74.2 |
| | Large Yellow | 0.002 | 0.0 | 20.0 | 10 | 0.9 | -91.2 |
| | Small Yellow | 0.002 | 0.2 | 85.0 | 10 | 4.2 | -58.1 |
| | Large Hyaline | 0.002 | 0.1 | 35.0 | 10 | 1.8 | -82.3 |
| | Small Hyaline | 0.002 | 0.3 | 140.0 | 10 | 6.8 | -31.9 |
| | Black | 0.002 | 0.4 | 165.0 | 10 | 8.5 | -15.1 |
| | Green | 0.002 | 0.0 | – | 10 | 0.0 | -100.0 |
| | Total | 0.02 | 4.2 | 208.5 | 100 | 100.0 | 0.0 |

* Plants were grown under prairie conditions.

The lack of spore reproduction in so many pots inoculated with a single spore (Table 5.11) was caused in part by the very poor condition of the U of MN growth chambers. The growth chambers used for corn, side-oats grama, and big bluestem inoculated plants all had various problems. The chambers used for the corn shut-off for a brief period in December, 1994, and overheated and shut-off in March, 1995. Because of problems in keeping the chambers running

properly, the big bluestem had to be moved to three different chambers. The U of MN has solved the growth chamber problems by purchasing new chambers with an anticipated summer, 1996 installation date.

During the production of the AMF inocula a number of problems were identified and addressed that will make production of the inocula easier in the future. Most of the soil used was steam sterilized by means of the CBS Greenhouse methods of a single treatment. Upon AMF spore isolation, this soil was found to contain a few spores that may have been viable. The problem of soil sterility has been solved for any soil used in an inoculum production set-up by sterilizing the soil three times and cooling it between treatments.

How to prevent outside AM spore contamination during inoculum production is a difficult problem. Using filter paper or a coffee filter to cover the surface of a pot was not effective. Degradation of the filter paper occurred after several weeks. The coffee filter's hole, made for the plant to grow through, was kept small to reduce contamination, but it also reduced plant growth, since the tillers that grew under the filter soon died due to lack of sunlight. Aluminum foil used in some experiments greatly increased the wetness of the soil and likely reduced AMF spore formation. Beads, as an alternative surface cover, will be tried in some future experiments.

The use of an automated/experimental irrigation method (see Chapter 6) is a promising addition to the traditional way of manually watering set-ups. It is anticipated that this irrigation system will generate more spores of different kinds than the traditional method.

# CHAPTER 6. ARBUSCULAR MYCORRHIZA IN PLANT INTERACTIONS WITH HEAVY METALS-Reproduction of AMF strains to be used in investigations on mechanisms of heavy metal tolerance in AM and restoration practices

## 6.1 Overview

The AM fungi are obligate plant symbionts [22]. Therefore, isolation of individual species and establishment of clonal cultures, which will serve as a source of inoculum for further experiments and restoration practices, has to be preceded by establishment of single spore inoculated host plant pot cultures [40]. Three different approaches to propagate AM fungal spores were tested: single spore inoculated pot cultures in traditional hand watering system, single spore inoculated pot cultures in automated irrigation system using principles of the Beltsville method [41] and *in vitro* dual cultures consisting of excised plant roots colonized by AM fungus [42]. Development of *in vitro* dual cultures seems to be especially promising. These cultures not only constitute an excellent system for physiological studies on AM [42] but also create source of single spore inoculum which can be used for pot cultures, providing a much higher chance of successful colonization of plant roots. This is not always the case in pot cultures established by placing a single spore on plant roots. Three different excised root systems were tested: monocot roots (corn), and dicot roots (tomato and carrot). We attempted to develop genetically modified carrot roots by infecting them with *Agrobacterium rhizogenes* inducing tumoral hairy root formation (Ri T-DNA transformed roots). Hairy roots have bacterial Ri T-DNA integrated into their chromosomes which induces high growth rate and low nutritional requirements suitable for inoculating them by AM fungi [42].

## 6.2 Materials and Methods

### 6.2.1. Pot cultures in traditional hand watering system

Spores of AM fungi isolated from heavy metal contaminated soil associated with roots of corn *(Zea mays* L.*)* plants collected from the landfill site (see Materials and Methods in Chapter 4) as well as from rhizosphere of corn plants grown on the experimental field of the U of MN (for

control) were segregated according to their color and size which is correlated with species. Single spores were used to inoculate roots of 7 day old seedlings of hybrid corn germinated under aseptic conditions. Spores were placed on the roots. Two potting media were used to plant inoculated seedlings as well as non inoculated control plants. One medium was 1:1 soil-sand mix consisting of heat sterilized soil provided by the CBS greenhouse and 2:1 mixture of coarse and fine autoclaved sand. After planting, pots were placed in the greenhouse and watered with deionized water as needed. The total number of plants was 91. The second medium was autoclaved sand (2:1 mixture of coarse and fine sand). After planting, pots were placed in a growth chamber and watered every other day with a half strength, modified Hoagland's nutrient solution plus added phosphate [41]. Since the corn plants exhibited growth retardation in comparison to plants grown in soil-sand mix, after about a month, half of the plants were given additional nutrients when watered. The total number of plants was 40. After about 4 months of growth, naturally senesced corn plants were harvested in the fall.

### 6.2.2. Pot cultures in automated irrigation system

An automated drip irrigation system [41] for reproduction of spores of AM fungi was established at the U of MN in the CBS greenhouse. The irrigation system consisted of 1.9 cm internal diameter irrigation polypipe (Eagle Plastic Inc. Hastings, Nebraska) with Dramm Dribble Rings® which were placed on the surface of potting medium in each pot (see Figure 3 by D. Stenlund, a modification of the figure in Millner and Kitt [41]). The number of pots attached to the irrigation system can be easily adjusted. The system is controlled by a digital weekly time switch (Omron model H5S), which is connected to a submersible pump (Little Giant model 2E-N), and a recirculating submersible pump (Little Giant model P-AAA), which is placed in 114 l covered nutrient reservoir. The submersible pump delivers watering solution to the pots at the set time; whereas the recirculating pump mixes solution prior to each watering event.

To test the functioning of the irrigation system, big bluestem (*Andropogon gerardii* Vitman) plants inoculated with single spores of AM fungi isolated from heavy metal contaminated soil associated with roots of corn plants collected from the landfill site were used. Big bluestem seeds

were germinated in vermiculite-perlite mix (3:1). Clusters of plants were inoculated with a single spore and placed in 5 1/4" pots containing greenhouse garden soil-sand mix (1:1). Garden soil was steamed three times for two hours in 48 hours intervals. Sand was autoclaved twice for 1 hour at 121°C. Plants of two different ages were used: 7 and about 50-days old. A total of 80 pots were established. Plants were irrigated automatically with 190 ml of deionized water delivered every day at 7 a.m. Since the plants were waterlogged, after about a month, the watering system was adjusted several times resulting in an optimal watering schedule: 20 ml of water per pot two times a day: at 7 a.m. and 1 p.m. (40 ml per day total). This watering schedule eliminated the problem of water logged soil and promoted plant growth.

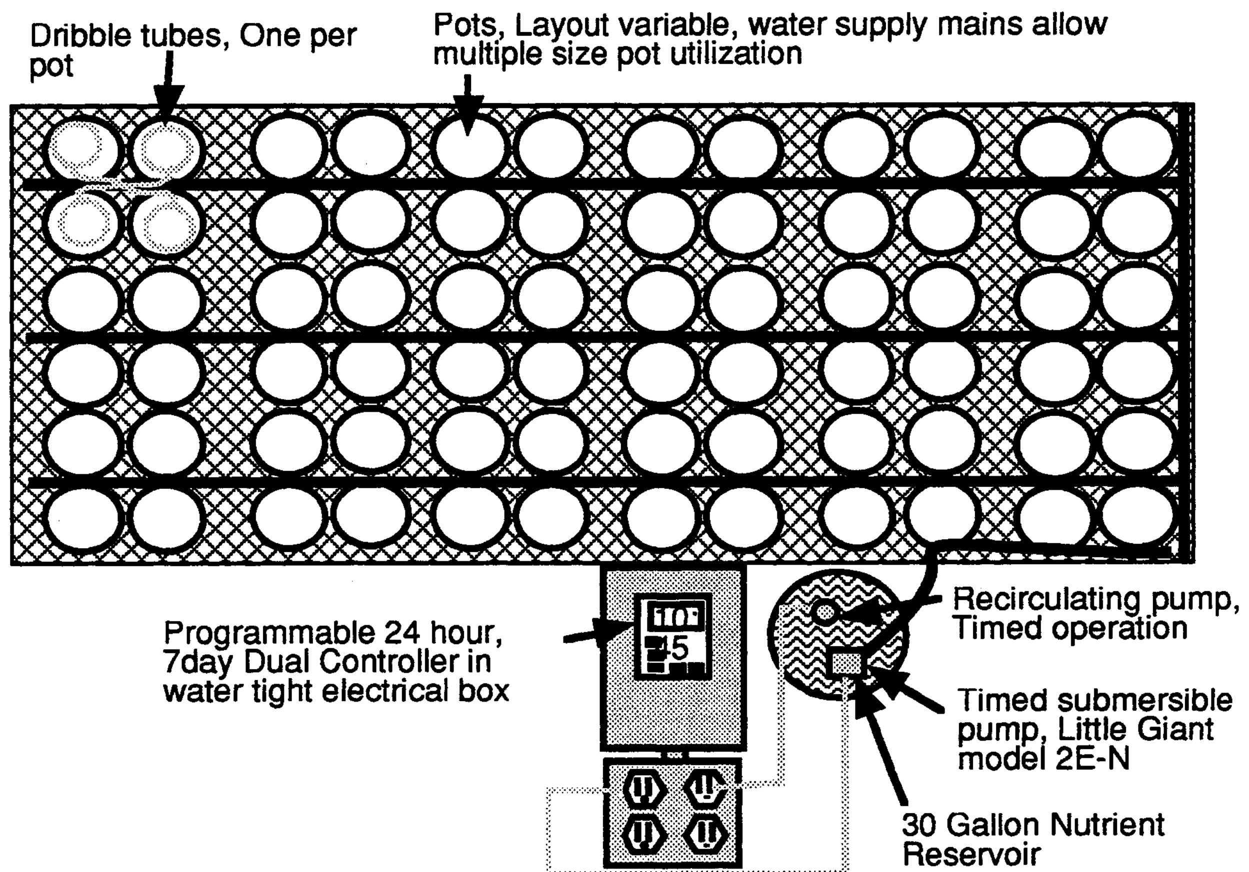


Figure 6.3.Automatic plant water/nutrient delivery system for the production of arbuscular mycorrhizal inoculum.

### 6.2.3. *In vitro* dual cultures

#### 6.2.3.1. Establishment of corn and tomato root cultures

To establish corn *(Zea mays)* root cultures, seeds from hybrid lines were surface sterilized with one of two different sterilizing agents: 5% sodium hypochlorite for 30 minutes with stirring or 30% $H_2O_2$ for 30 minutes with stirring. After imbibition in sterile deionized water for 24 hours seeds were placed on filter paper and left to germinate in darkness.

To establish tomato (*Lycopersicon esculentum* L. var. Beefsteak) root cultures, seeds were surface sterilized in 30% $H_2O_2$ for 15 minutes and rinsed 3 times in sterile deionized water. Sterile seeds were placed on water agar to germinate.

After germination, 0.5 to 3 cm roots segments of developing corn or tomato seedlings were excised and placed on agar modified White's medium [42]. They were grown in darkness in thermoperiod 14 D : 10 N at 25 : 20°C.

#### 6.2.3.2. Establishment of Ri T-DNA transformed carrot roots

Carrot roots were cut into 10 - 12 cm long pieces. Orientation of each piece was marked. Root fragments were washed in soapy water, rinsed with 95% ethanol and sterilized with 30% $H_2O_2$ + Tween 20 for 15 minutes with manual shaking. Two cm long fragments from each side of each piece were discarded and the remaining fragments were sliced into 0.5 cm thick slices and trimmed on sides (about 2-3 mm of root cortex was removed). Slices were placed on 1% water agar and inoculated with one loop full of 30 hours old culture of *Agrobacterium rhizogenes* A4TC24 Rif R grown in YMB liquid medium [43].

#### 6.2.3.3. Sterilization procedure for AMF spores to be used in dual cultures

AMF spores were isolated from nature using wet sieving and sucrose centrifugation (Appendix C). Healthy looking spores were collected from the filters and segregated according to color and size. For sterilization, spores were washed from filters with solution of antibiotics containing Tween 20 into 1.5 ml eppendorf tubes, rinsed in Tween 20 and soaked in 2% chloramine T for 1 hr. After rinsing with deionized water, spores were soaked 3 times for 0.5 hr

in deionized water and stored in a solution of antibiotics at 4°C. Thus prepared spores were used to inoculate excised corn roots or transferred on 1% water agar for storage or germination.

## 6.3 Results and Discussion

### 6.3.1. Pot cultures in traditional hand watering system

Comparison of spore production by fungi associated with plants grown in soil-sand mix and in sand watered with Hoagland's solution showed that soil-sand mix was more effective in supporting AMF spore production. The reproduction of AM spores in association with plants grown in sand medium was achieved in only three out of 35 pots indicating that the method has to be adjusted. Plants grown in the soil-sand mix yielded between 1 and 184 AMF spores per gram of dry potting medium. Figure 4 presents percentages of spore types reproduced in soil-sand medium depending on type and origin of spore inoculum. Presence of black spores can be attributed to the persistence of the thick cell wall of this spore type in heat sterilized soil.

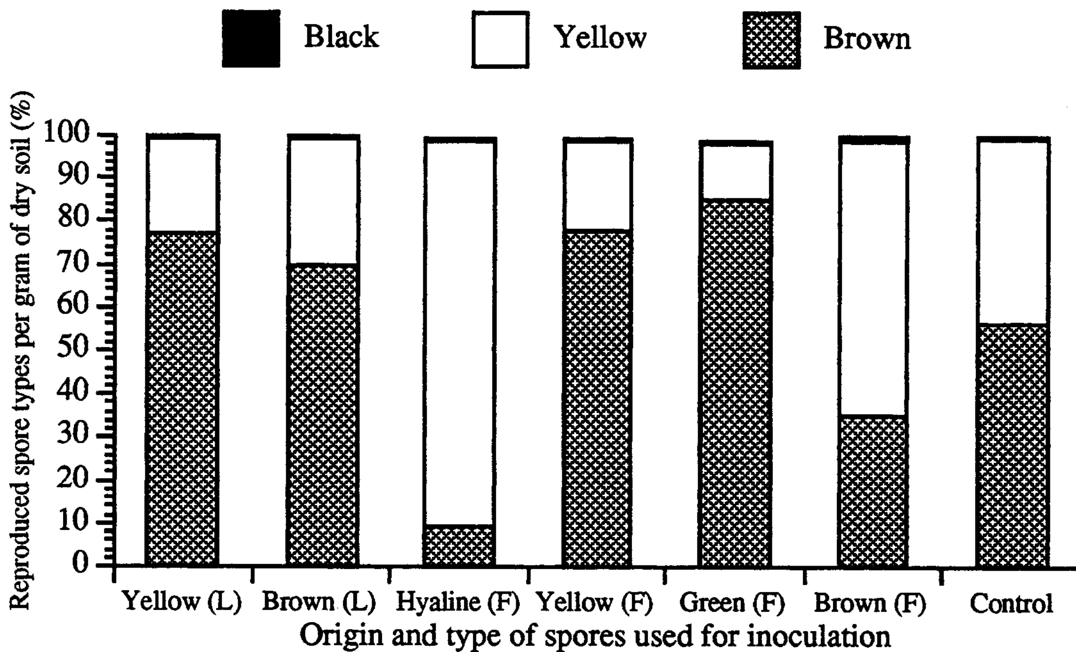


Figure 6.4. Spore types obtained from single spore inoculation of corn plants grown in soil-sand mix. L - landfill contaminated with heavy metals; F - corn field.

To confirm this, spore germination tests should be performed. In many cases the single spore cultures were cross-contaminated. This indicated that special precautions should be taken to

ascertain that spores are not transferred between pots or introduced from the environment, which was the case under the greenhouse conditions. Nonetheless, successful reproduction of AM fungal spores created uniform populations of AM fungi, which will be used in further experiments.

### 6.3.2. Pot cultures in automated irrigation system

AMF spore propagation experiment will be terminated and completed by fall, 1995. Additional spore propagation experiments will be done using this system. Further testing of the automated drip irrigation system will include delivery of nutrient solution to AMF inoculated and non inoculated plants grown in a sand medium.

### 6.3.3. *In vitro* dual cultures

#### 6.3.3.1. Establishment of corn and tomato root cultures

Successful establishment of corn root cultures was obtained in one of the two hybrid lines. Established corn and tomato root cultures are maintained on modified White's agar medium [42] and can be inoculated with AM fungi. It seems that excised corn roots are not very suitable for maintenance of AM fungi in dual plant cultures: they have a very low growth rate and cease growing after only a few rounds of subculturing. Tomato roots on the other hand, are very easy to maintain and subculture.

#### 6.3.3.2 Establishment of Ri T-DNA transformed carrot roots

After more than a month, appearance of scarce fibrous roots was observed on some carrots discs indicating that probably a different *Agrobacterium* strain should be used to induce formation of fibrous roots in carrots.

#### 6.3.3.3. Sterilization procedure for AMF spores to be used in dual cultures

The applied sterilization procedure of AMF spores appeared to be sufficient to obtain sterile spores to be used in establishment of non contaminated dual cultures of AM roots. Spores remained sterile after one year long storage at 4°C.

# CHAPTER 7. AM RE-INTRODUCTION

## 7.1 Overview

Arbuscular mycorrhizal fungi benefit plants by increasing the uptake of nutrients [44]. It has also been shown that the presence of AMF can inhibit the germination of weedy, non-mycotrophic species, increase plant diversity [45] and increase the competitive effects of native prairie species [46]. It has also been shown that the effects that AMF have on plant communities are especially pronounced at highly disturbed sites [47]. For these reasons, AMF are an essential component of roadside prairie restorations.

Given the nature of highly disturbed roadside environments, it may become necessary to re-introduce AMF into the soil when seeding. In order to quantify the effects of AMF re-introduction, an experiment was initiated wherein AMF inoculum produced in the greenhouse was incorporated into the soil with the prairie seed in a roadside planting.

## 7.2 Materials and Methods

### 7.2.1 Laboratory Procedures

Inoculum was produced by the procedures outlined in Section 5.2.1 for general inoculum. Inoculum produced from little bluestem (*Schizacharyium scoparium* (Michx.) Nash) was chosen as the most appropriate inoculum because of the high spore numbers (Table 5.10) and because the original source of those spores was the Crosstown prairie remnant. The Crosstown prairie is located in the Twin Cities Metro area and is a native prairie containing a large amount of mycorrhizal species diversity. Inoculum was prepared as outlined by Stenlund *et al.* [7]. The inoculum was sieved through a 1cm hardware cloth to break up large soil fragments and grind up the root mass. Roots that could not be ground up were cut into 1cm strips. The inoculum was thoroughly homogenized and placed in cold storage until ready for use.

The sterilized greenhouse soil was treated with a microbial rinse from the inoculated soil [49]. This was done to ensure that beneficial microbes present in the inoculum were also present in the sterilized soil control. Approximately 2 kilograms of dry inoculum soil were rinsed with 2

liters of water to yield 1 liter of microbial water. This water was then added to the 17 kg of sterilized greenhouse soil.

Seed was obtained from Prairie Restorations Inc., Princeton, MN. Two of their seed mixes were used: Mixed Height/ Mesic Grass Mix and Mixed Height/ Mesic Wildflower Mix. The plant species present are listed in Table 7.12. These two mixes were then combined at a ratio of 4:1 grass to forb by weight. These mixes were chosen because they are similar to the mix that Mn/DOT used at the Cambridge prairie restoration complex.

Table 7.12. Percentages of species present in the seed mix planted at the JES restoration site

| Percent | Common Name | Latin Name |
|---|---|---|
| Grasses* | | |
| 26.0% | Big blue stem | *Andropogon gerardii* Vitman |
| 19.0% | Little blue stem | *Schizachyrium scoparium* (Michx.) Nash |
| 19.0% | Indian grass | *Sorgastrum nutans* (L.) Nash |
| 6.8% | Side oats grama | *Bouteloua curtipendula* (Michx.) Torr. |
| 1.8% | Canada wild rye | *Elymus canadensis* L. |
| 1.8% | Switch grass | *Panicum virgatum* L. |
| Forbs** | | |
| 3.3% | Black-eyed susan | *Rudbeckia hirta* L. |
| 3.0% | Azure aster | *Aster oolentangiensis* Riddell |
| 3.0% | Purple prairie clover | *Petalostemum purpureum* (Vent.) Rybd. |
| 2.0% | White prairie clover | *Petalostemum candidum* (Willd.) Michx. |
| 1.5% | Common ox-eye | *Heliopsis helianthoides* (L.) Sweet |
| 1.3% | Giant hyssop | *Agastache foeniculum* (Pursh) Kuntze |
| 1.3% | Butterfly weed | *Asclepias tuberosa* L. |
| 1.3% | Wild bergamot | *Monarda fistulosa* L. |
| 1.0% | Bush clover | *Lespedeza capitata* Michx. |
| 1.0% | Stiff goldenrod | *Solidage rigida* L. |
| 1.0% | Hoary vervain | *Verbena stricta* Vent. |
| 1.0% | Golden alexander | *Zizia aurea* (L.) Koch |
| 0.5% | Yarrow | *Achillea millifolium* L. |
| 0.5% | Leadplant | *Amorpha canescens* Pursh |
| 0.5% | Stiff tickseed | *Coreopsis palmata* Nutt. |
| 0.5% | Northern bedstraw | *Galium boreale* L. |
| 0.5% | Tall blazingstar | *Liatris pycnostachya* Michx. |
| 0.5% | Gray goldenrod | *Solidago nemoralis* Aiton |
| 0.5% | Showy goldenrod | *Solidago speciosa* Nutt. |

* Percentages by pure live seed (PLS) weights

** Percentages by bulk weights

The following seeds were present in amounts < 0.5%: Kalm's Brome (*Bromus kalmii* A. Gray), Prairie sunflower (*Helianthus petiolaris* Nutt.), Stiff sunflower (*Helianthus rigidus* (Cass.) Desf., Prairie rose (*Rosa arkansana* T. C. Porter) and Upland goldenrod (*Solidago ptarmicoides* (Neas) B. Boivin).

Inoculum, sterilized soil and seeds were all weighed out in the lab prior to field use to ensure an even distribution within and throughout the plots. Three hundred fifty grams of sterilized soil and inoculum were weighed out for placement in each row. This equals a rate of application of approximately 3.9 tons per acre of inoculum. The seed mix was weighed out on a per plot basis because the weights were to small to obtain accurate measurements on a per row basis. The seed was then divided up for the individual rows in the field. The weights used were as follows: .567 grams of seed per row, 2.84 grams of seed per plot, and 27 lbs of seed per acre.

### 7.2.2. Field Procedures

The field plots at the JES restoration site are approximately 50 meters northwest of the JES pond. This site was chosen because the level surface reduces the chance of erosion in comparison to the steep slopes directly surrounding the pond. The entire area used for experimental purposes is $288m^2$. The area consists of 24 individual 2m x 1m plots with a 2m buffer zone surrounding each plot (Figure 7.5). Randomly placed in each of the 24 plots was one of three treatments: seed only (control), seed and sterilized greenhouse soil (control), and seed and mycorrhizal inoculum. This resulted in 8 plots per treatment. In each treatment, four of the eight plots will be used to measure aboveground parameters and four will be used to measure below ground parameters.

The possibility of employing the seed drill used by Mn/DOT was discussed at length with Mr. Robert Jacobson. It was determined that in order to quantify exactly how much inoculum and seed was being planted the best alternative is to plant by hand. This was necessary in order to obtain meaningful experimental results. If the results show significant improvement with AM inoculum, seeding with inoculum directly from the seed drill is possible and may yield similar results.

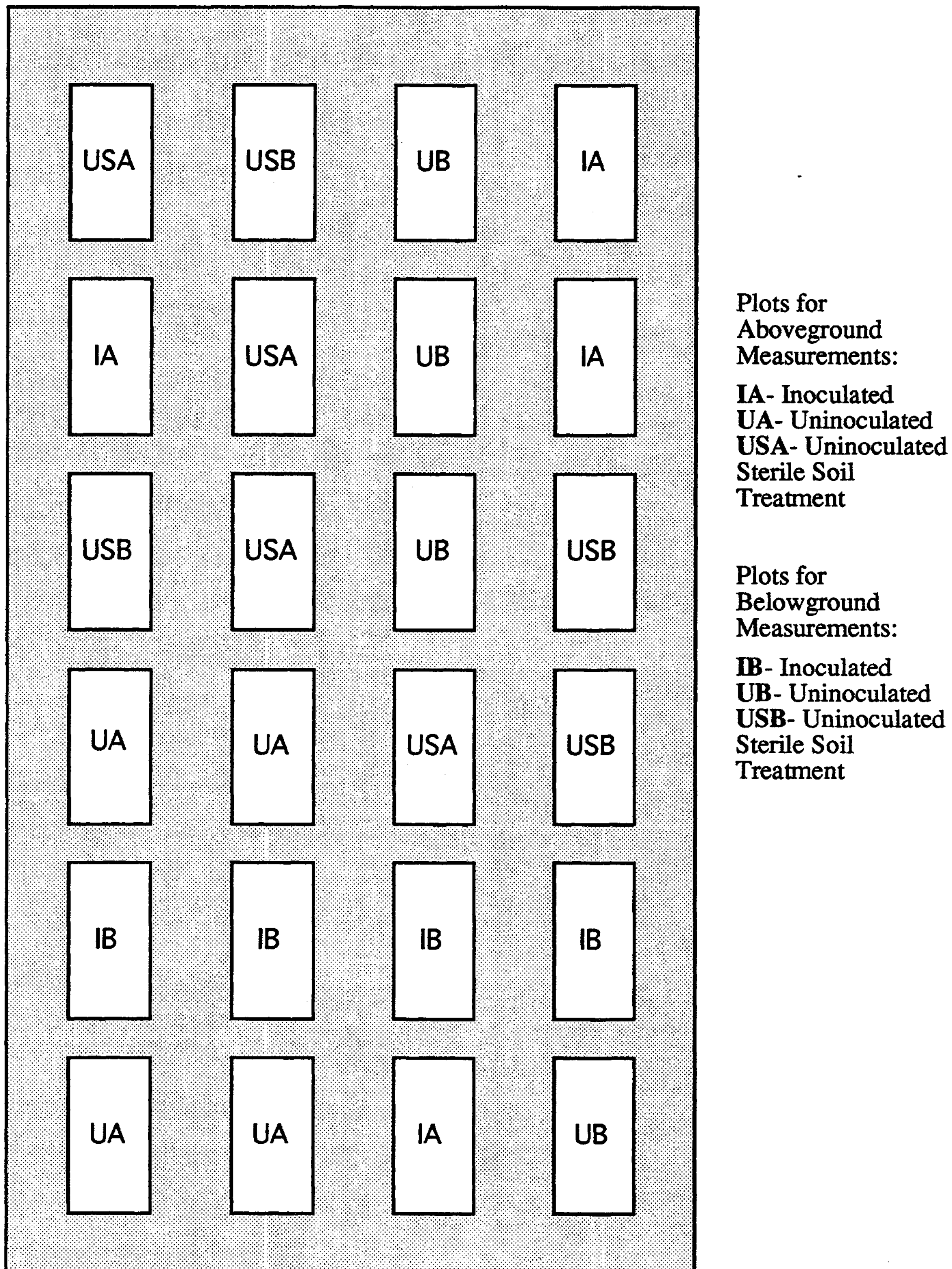


Figure 7.5. Experimental plots established at the JES restoration site in Cambridge, MN.

Furrows were made by means of a belt seeder (University of Minnesota, Department of Agronomy, 1957) approximately 1/2 inch deep. The depth was chosen such that final seed depth was 1/4 inch as recommended by Prairie Moon Nursery, Winona, MN. In each plot, 5 furrows were made lengthwise at approximately 8 inch spacing. This spacing was chosen because it is standard practice for Mn/DOT when seeding these native prairie species. In order to obtain even distribution in the individual furrows of inoculum, sterile soil and seed, these weighed samples were placed into one half of a 4 inch diameter PVC pipe and spread evenly before placing in the furrows. Sterile soil and inoculum were placed below the seed in order to maximize root contact with mycorrhizae. Furrows were then covered with soil from the site and patted down to ensure adequate seed to soil contact.

## 7.3 Results and Discussion

As mentioned above, planting was performed in such a way that precise weights of seed and inoculum is known. This will allow us to draw conclusions concerning the amount of inoculum and seed needed to obtain desired results. Given that the plots were seeded in late spring, 1995 such conclusions at this point would be premature. As shown in Table 7.13, the site is still in the early stages of succession, having very low numbers of mycorrhizal spores per gram dry soil.

Table 7.13: Mycorrhizal Spore Number and Diversity at Time of Planting of Experimental JES Prairie Restoration Plots

| Spore Color | Large Brown | Small Brown | Large Red | Small Red | Large Yellow | Small Yellow | Small Hyaline | Green | TOTAL |
|---|---|---|---|---|---|---|---|---|---|
| JES Plots | 0.1±0.0 | 3.7±1.1 | 0.1±0.0 | 0.6±0.3 | 0.0±0 | 0.1±0.0 | 0.2±0.1 | 0.6±0.4 | 5.2±1.2 |

Early succession is also illustrated in Table 7.14 which shows that the site is still dominated by weedy, non-planted species. The presence of so many planted species is, however, quite promising.

Table 7.14: A List of Plant Species Recorded at the JES Experimental Prairie Restoration Plots

| **Planted Grasses and Forbs** | |
|---|---|
| *Latin Name* | Common Name |
| *Andropogon gerardii* Vitman | big blue stem |
| *Schizachyrium scoparium* (Michx.) Nash | little blue stem |
| *Bouteloua curtipendula* (Michx.) Torr. | side-oats grama |
| *Rudbeckia hirta* L. | black eyed susan |
| *Heliopsis helianthoides* (L.) Sweet | early sunflower |
| *Regreen* | regreen |
| *Triticum aestivum* L. | slender wheat |
| *Lolium* spp. | annual rye |
| | |
| **Non-Planted Grasses and Forbs** | |
| *Latin Name* | Common Name |
| *Setaria glauca* (L.) P. Beauv. | yellow foxtail |
| *Trifolium pratense* L. | red clover |
| *Fragaria* spp. | strawberry |
| *Silene pratense* (Refn) Godron & Gran. | bladder campion |
| *Vicia sativa* L. | vetch |
| *Digitaria* spp. | crab grass |
| *Erigeron canndensis* L. | fleabane |
| *Trifolium* spp. | yellow clover |
| *Medicago sativa* L. | alfalfa |
| *Ambrosia artemisiifolia* L. | ragweed |
| *Polygonum* spp. | smart weed |
| *Brassica* spp. | mustard |
| *Melilotus alba* Medikus | white clover |
| *Oxalis acetosella* L. | wood sorrel |
| *Plantago major* L. | plantain |

Table 7.15 lists the plant species that are dominant in the seed mix and shows the current status of those species as of 9/12/95. The three dominant grasses (*Andropogon gerardii, Schizachyrium scoparium,* and *Bouteloua curtipendula*) and two of the five dominant forbs (*Rudbeckia hirta* and *Heliopsis helianthoides*) had all germinated at that time. Given favorable growing conditions next year, more conclusions may be drawn concerning any differences in germination and/or growth of the plants in the different experimental plots.

Table 7.15. Percent Germination of Dominant Seed Species Planted at JES Prairie Restoration Site, Cambridge, MN

| Seed Species | | Germinated by 9/12/95 |
|---|---|---|
| Latin Name | Common Name | |
| *Andropogon gerardii* Vitman | big blue stem | Yes |
| *Schizachyrium scoparium* (Michx.) Nash | little blue stem | Yes |
| *Bouteloua curtipendula* (Michx.) Torr. | side-oats grama | Yes |
| *Sorghastrum nutans* (L.) Nash | indian grass | No |
| *Rudbeckia hirta* L. | black eyed susan | Yes |
| *Heliopsis helianthoides* (L.) Sweet | early sunflower | Yes |
| *Petalostemum purpureum* (Vent.) Rybd. | purple prairie clover | No |
| *Petalostemum candidum* (Willd.) Michx. | white prairie clover | No |
| *Aster oolentangiensis* Riddell | azure aster | No |
| | **Total Percent Germination** | **56%** |

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**Appendix A. Plant species lists from prairie and wetland sites compiled between September and November 1994.**

__Remnant Prairie__
**Crosstown Prairie**

| Plant Family | Scientific Name | Common Name(s) |
|---|---|---|
| **Aceraceae** | *Acer negundo* | Boxelder |
| **Asclepiadaceae** | *Asclepias syrica* | Common milkweed |
| **Asteraceae** | *Achillea millefolium* | Common yarrow |
| | *Ambrosia* sp. | Ragweed |
| | *Artemesia* sp. | Sage |
| | *Aster* sp. | Aster |
| | *Liatrus* sp. | Blazing star |
| | *Solidago* sp. | Goldenrod |
| | *Solidago rigida* | Stiff goldenrod |
| **Equisetaceae** | *Equisetum* sp. | Scouring rush |
| **Euphorbiaceae** | *Euphorbia* sp. | Spurge |
| **Fabaceae** | *Lotus corniculatus* | Birdsfoot-trefoil |
| **Lamiaceae** | *Monarda fistulosa* | Wild bergamot |
| **Oleaceae** | *Fraxinus* sp. | Ash |
| **Onagraceae** | *Oenothera biennis* | Evening-primrose |
| **Poaceae** | *Andropogon gerardii* | Big bluestem |
| | *Panicum* sp. | Panic-grass |
| | *Schizachyrium scoparium* | Little bluestem |
| | *Setaria* sp. | Foxtail grass |
| | *Sorghastrum nutans* | Indian grass |
| **Polygonaceae** | *Polygonum* sp. | Smartweed, knotweed |
| **Rosaceae** | *Rosa* sp. | Rose |
| | *Rubus* sp. | Bramble |
| **Rubiaceae** | *Galium* sp. | Bedstraw, cleavers |
| **Scrophulariaceae** | *Veronicastrum virginicum* | Culver's root |
| **Solanaceae** | *Solanum dulcamara* | Bittersweet nightshade |

__Restored Prairie__
**Lake Shetek**

| Plant Family | Scientific Name | Common Name(s) |
|---|---|---|
| **Asclepiadaceae** | *Asclepias* sp. | Milkweed |
| **Asteraceae** | *Achillea millefolium* | Common yarrow |
| | *Aster* spp. | Asters |
| | *Cirsium* sp. | Thistle |
| | *Helianthius* sp. | Sunflower |
| | *Ratibida* sp. | Coneflower |
| | *Rudbeckia hirta* | Black-eyed Susan |
| | *Zizia* sp. | Golden alexanders |
| **Fabaceae** | *Lotus corniculatus* | Birdsfoot-trefoil |
| | *Melilotus alba* | White sweet clover |
| **Lamiaceae** | *Monarda fistulosa* | Wild bergamot |
| **Onagraceae** | *Oenothera* sp. | Evening-primrose |
| **Poaceae** | *Andropogon gerardii* | Big bluestem |
| | *Bouteloua curtipendula* | Side-oats grama |
| | *Elymus canadensis* | Canada wild rye |
| | *Panicum virgatum* | Switchgrass |

| Plant Family | Scientific Name | Common Name(s) |
|---|---|---|
| | *Phleum pratense* | Timothy |
| | *Spartina pectinata* | Prairie cord-grass |
| **Polygonaceae** | *Polygonum* sp. | Smartweed, knotweed |

**Materials and Research Laboratory (Mn/DOT)**

| Plant Family | Scientific Name | Common Name(s) |
|---|---|---|
| **Asteraceae** | *Achillea millefolium* | Common yarrow |
| | *Arctium* sp. | Burdock |
| | *Artemesia ludoviciana* | Prairie sage |
| | *Aster ericoides* | Heath aster |
| | *Aster laevis* | Smooth-blue aster |
| | *Aster novae-angliae* | New England aster |
| | *Centaurea* sp. | Knapweed |
| | *Ratibida* sp. | Coneflower |
| | *Solidago* sp. | Goldenrod |
| | *Solidago rigida* | Stiff goldenrod |
| **Brassicaceae** | *Berteroa incana* | Hoary allysum |
| **Equisetaceae** | *Equisetum hyemale* | Scouring rush |
| | *Equisetum pratense* | Meadow-horsetail |
| **Fabaceae** | *Coronilla varia* | Crown-vetch |
| | *Melilotus alba* | White sweet clover |
| | *Melilotus officinalis* | Yellow sweet clover |
| | *Trifolium pratense* | Red Clover |
| **Lamiaceae** | *Agastache foeniculum* | Lavendar giant hyssop |
| | *Lycopus* sp. | Water-horehound |
| | *Monarda fistulosa* | Wild bergamot |
| **Oxalidaceae** | *Oxalis* sp. | Wood-sorrel |
| **Poaceae** | *Andropogon gerardii* | Big bluestem |
| | *Bouteloua curtipendula* | Side-oats grama |
| | *Elymus canadensis* | Canadian wild rye |
| | *Eragrostis spectabilis* | Purple lovegrass |
| | *Muhlenbergia racemosa* | Muhly |
| | *Phalaris arundinacea* | Reed canary grass |
| | *Schizachyrium scoparium* | Little bluestem |
| | *Setaria viridis* | Green foxtail |
| | *Sorghastrum nutans* | Indian grass |
| | *Spartina pectinata* | Prairie cord-grass |
| | *Triticum aestivum* | Common wheat |
| **Polygonaceae** | *Polygonum* sp. | Smartweed, knotweed |
| **Rosaceae** | *Rosa* sp. | Rose |
| **Scrophulariaceae** | *Linaria vulgaris* | Butter-and-eggs |
| | *Verbascum thapsus* | Common mullein |
| **Ulmaceae** | *Ulmus pumila* | Siberian elm |
| **Verbenaceae** | *Verbena* sp. | Vervain |

**St. Croix Rest Area (Mn/DOT - Burned Site)**

| Plant Family | Scientific Name | Common Name(s) |
|---|---|---|
| **Anacardiaceae** | *Rhus* sp. | Sumac |
| **Asteraceae** | *Aster novae-angliae* | New England aster |
| | *Centaurea* sp. | Knapweed |
| | *Ratibida* sp. | Coneflower |
| | *Solidago rigida* | Stiff goldenrod |

| | | |
|---|---|---|
| **Brassicaceae** | *Berteroa incana* | Hoary allysum |
| **Fabaceae** | *Coronilla varia* | Crown-vetch |
| | *Dalea purpurea* | Purple prairie-clover |
| | *Melilotus alba* | White sweet clover |
| | *Trifolium pratense* | Red Clover |
| **Lamiaceae** | *Monarda fistulosa* | Wild bergamot |
| **Poaceae** | *Andropogon gerardii* | Big bluestem |
| | *Bouteloua curtipendula* | Side-oats grama |
| | *Panicum virgatum* | Switchgrass |
| | *Schizachyrium scoparium* | Little bluestem |
| | *Sorghastrum nutans* | Indian grass |
| **Rosaceae** | *Pyrus* sp. | Crab-apple |
| **Salicaceae** | *Populus deltoides* | Cottonwood |
| **Ulmaceae** | *Ulmus pumila* | Siberian elm |

**St. Croix Rest Area (Mn/DOT - Unburned Site)**

| Plant Family | Scientific Name | Common Name(s) |
|---|---|---|
| **Asteraceae** | *Cirsium vulgare* | Bull-thistle |
| **Poaceae** | *Andropogon gerardii* | Big bluestem |
| | *Bromus* sp. | Brome-grass |
| | *Panicum virgatum* | Switchgrass |
| | *Schizachyrium scoparium* | Little bluestem |
| **Ulmaceae** | *Ulmus pumila* | Siberian elm |

**University of Minnesota Horticulture Research Prairie**

| Plant Family | Scientific Name | Common Name(s) |
|---|---|---|
| **Apiaceae** | *Eryngium yuccifolium* | Rattlesnake-master |
| **Asclepiadaceae** | *Asclepias incarnata* | Swamp-milkweed |
| | *Asclepias tuberosa* | Butterfly-weed |
| **Asteraceae** | *Artemesia* sp. | Sage |
| | *Aster ericoides* | Squarrose white aster |
| | *Aster oolentangiensis* | Prairie heart-leaved aster |
| | *Aster novae-angliae* | New England aster |
| | *Aster pilosus* | Awl-aster |
| | *Aster praealtus* | Veiny lined aster |
| | *Coreopsis palmata* | Finger tickseed |
| | *Echinacea* sp. | Coneflower |
| | *Euthamia graminifolia* | Flat-topped goldenrod |
| | *Helianthus* sp. | Sunflower |
| | *Heliopsis helianthoides* | Sunflower-everlasting |
| | *Liatrus* sp. | Blazing star |
| | *Liatrus aspera* | Lacerate blazing star |
| | *Ratibida pinnata* | Coneflower |
| | *Rudbeckia lacinata* | Cutleaf-coneflower |
| | *Silphium integrifolium* | Prairie rosin-weed |
| | *Silphium laciniatum* | Compass-plant |
| | *Silphium perfoliatum* | Cup-plant |
| | *Silphium terebinthinaceum* | Basal-leaved rosin-weed |
| **Clusiaceae** | *Hypericum* sp. | St. John's-wort |
| **Commelinaceae** | *Tradescantia* sp. | Spiderwort |
| **Fabaceae** | *Amorpha canescens* | Lead-plant |
| | *Desmodium* sp. | Tick-trefoil |

| | | |
|---|---|---|
| **Gentianaceae** | *Gentiana andrewsii* | Bottle-gentian |
| **Lamiaceae** | *Agastache foeniculum* | Lavendar giant hyssop |
| | *Pycnanthemum* sp. | Mountain-mint |
| **Poaceae** | *Andropogon gerardii* | Big bluestem |
| | *Bouteloua curtipendula* | Side-oats grama |
| | *Panicum virgatum* | Switchgrass |
| | *Schizachyrium scoparium* | Little bluestem |
| | *Sorghastrum nutans* | Indian grass |
| | *Spartina pectinata* | Prairie cord-grass |
| **Ranunculaceae** | *Anemone* sp. | Anemone, windflower |
| | *Thalictrum* sp. | Meadow-rue |
| **Rosaceae** | *Rosa arkansana* | Dwarf prairie-rose |
| **Rubiaceae** | *Galium boreale* | Northern bedstraw |
| **Scrophulariaceae** | *Linaria vulgaris* | Butter-and-eggs |
| | *Penstemon* sp. | Beard-tongue |
| | *Veronicastrum virginicum* | Culver's root |
| **Saxifragaceae** | *Heuchera richardsonii* | Prairie alum-root |
| **Verbenaceae** | *Verbena stricta* | Hoary vervain |

**Remnant Wetland**
**Cambridge - Country Club**

| Plant Family | Scientific Name | Common Name(s) |
|---|---|---|
| **Alismataceae** | *Sagittaria* sp. | Arrow-head |
| **Asteraceae** | *Bidens cernua* | Bur-marigold |
| | *Eupatorium perfoliatum* | Boneset |
| **Balsaminaceae** | *Impatiens* sp. | Touch-me-not |
| **Cornaceae** | *Cornus* sp. | Dogwood |
| **Cyperaceae** | *Carex comosa* | Sedge |
| | *Carex* spp. | Sedges |
| **Lamiaceae** | *Lycopus* sp. | Water-horehound |
| **Lemnaceae** | *Lemna* sp. | Duckweed |
| **Poaceae** | *Calamagrostis stricta* | Reed-grass |
| **Polygonaceae** | *Rumex* sp. | Dock |
| **Salicaceae** | *Salix fragilis* | Crack-willow |
| **Typhaceae** | *Typha angustifolia* | Narrow-leaved cat-tail |
| | *Typha glauca* | Common cat-tail |

**Restored Wetland**
**Cambridge - JES**

| Plant Family | Scientific Name | Common Name(s) |
|---|---|---|
| **Asclepiadaceae** | *Asclepias* sp. | Milkweed |
| **Asteraceae** | *Ambrosia* sp. | Ragweed |
| | *Arctium* sp. | Burdock |
| | *Bidens cernua* | Bur-marigold |
| | *Cirsium* sp. | Thistle |
| | *Hieracium* sp. | Hawkweed |
| | *Rudbeckia hirta* | Black-eyed Susan |
| | *Taraxacum officinale* | Common dandelion |
| **Brassicaceae** | *Berteroa incana* | Hoary alyssum |
| | *Capsella bursa-pastoris* | Shepherd's purse |
| **Caryophyllaceae** | *Silene* sp. | Catchfly, campion |
| **Cyperaceae** | *Carex* sp. | Sedge |

| | | |
|---|---|---|
| | *Carex comosa* | Sedge |
| | *Scirpus americanus* | Bulrush |
| **Equisetaceae** | *Equisetum* sp. | Scouring rush |
| **Fabaceae** | *Medicago lupulina* | Black medick |
| | *Trifolium hybridum* | Alsike clover |
| | *Trifolium pratense* | Red clover |
| | *Trifolium repens* | White clover |
| **Juncaceae** | *Juncus* sp. | Rush |
| **Lemnaceae** | *Lemna* sp. | Duckweed |
| **Onagraceae** | *Epilobium ciliatum* | American willow-herb |
| | *Oenothera* sp. | Evening-primrose |
| **Plantaginaceae** | *Plantago* sp. | Plantain |
| **Poaceae** | *Avena sativa* | Oats |
| | *Echinochloa crusgalli* | Barnyard-grass |
| | *Lolium perenne* var. *aristatum* | Italian ryegrass |
| | *Muhlenbergia* sp. | Muhly |
| | *Panicum virgatum* | Switchgrass |
| | *Phalaris arundinacea* | Reed canary grass |
| | *Phleum pratense* | Timothy |
| **Polygonaceae** | *Polygonum* sp. | Smartweed, knotweed |
| | *Rumex* sp. | Dock |
| | *Rumex acetosella* | Red sorrel |
| **Rosaceae** | *Potentilla norvegica* | Strawberry-weed |
| **Salicaceae** | *Populus deltoides* | Cottonwood |
| | *Salix nigra* | Black willow |
| **Scrophulariaceae** | *Verbascum thapsus* | Common mullein |
| **Typhaceae** | *Typha* sp. | Cat-tail |
| **Urticaceae** | *Urtica dioica* | Stinging nettle |
| **Verbenaceae** | *Verbena* sp. | Vervain |

**Inver Grove Heights - Island Pond**

| Plant Family | Scientific Name | Common Name(s) |
|---|---|---|
| **Asteraceae** | *Solidago* sp. | Goldenrod |
| **Brassicaceae** | *Berteroa incana* | Hoary alyssum |
| **Caryophyllaceae** | *Silene* sp. | Campion, catchfly |
| **Cucurbitaceae** | *Echinocystis lobata* | Wild cucumber |
| **Fabaceae** | *Trifolium* spp. | Clovers |
| **Fagaceae** | *Quercus macrocarpa* | Bur-oak |
| | *Quercus rubra* | Northern red oak |
| **Grossulariaceae** | *Ribes* sp. | Gooseberry, currant |
| **Lemnaceae** | *Lemna* sp. | Duckweed |
| **Malvaceae** | *Malva neglecta* | Common mallow |
| **Onagraceae** | *Oenothera biennis* | Evening-primrose |
| **Plantaginaceae** | *Plantago* sp. | Plantain |
| **Poaceae** | *Lolium perenne* var. *aristatum* | Italian ryegrass |
| | *Setaria* sp. | Foxtail grass |
| **Polygonaceae** | *Rumex* sp. | Dock |
| **Ranunculaceae** | *Clematis* sp. | Clematis |
| **Rhamnaceae** | *Rhamnus cathartica* | Common buckthorn |
| **Rosaceae** | *Potentilla* sp. | Cinquefoil |
| | *Rubus* sp. | Bramble |
| **Salicaceae** | *Populus deltoides* | Cottonwood |
| | *Populus tremuloides* | Quaking aspen |

| | | |
|---|---|---|
| **Scrophulariaceae** | *Verbascum thapsus* | Common mullein |
| **Ulmaceae** | *Ulmus americanus* | American elm |
| **Urticaceae** | *Urtica dioica* | Stinging nettle |

**Inver Grove Heights - Undisturbed Pond**

| Plant Family | Scientific Name | Common Name(s) |
|---|---|---|
| **Asteraceae** | *Achillea millefolium* | Common yarrow |
| | *Aster* sp. | Aster |
| | *Solidago* sp. | Goldenrod |
| **Brassicaceae** | *Berteroa incana* | Hoary alyssum |
| **Fabaceae** | *Trifolium* spp. | Clovers |
| **Lemnaceae** | *Lemna* sp. | Duckweed |
| **Liliaceae** | *Asparagus officinalis* | Asparagus |
| **Oxalidaceae** | *Oxalis* sp. | Wood-sorrel |
| **Plantaginaceae** | *Plantago* sp. | Plantain |
| **Poaceae** | *Avena sativa* | Oats |
| | *Lolium perenne* var. *aristatum* | Italian ryegrass |
| | *Setaria* sp. | Foxtail grass |
| **Rosaceae** | *Potentilla* sp. | Cinquefoil |
| | *Rubus* sp. | Bramble |
| **Scrophulariaceae** | *Verbascum thapsus* | Common mullein |
| **Typhaceae** | *Typha* sp. | Cat-tail |

**Appendix B. Types of transects done at the prairies and wetlands sampled throughout Minnesota.**

| Site | Date | Type of Transect | Plants Identified |
|---|---|---|---|
| Cambridge-Country Club | 7/2/94 | W-9mx10m | No |
| Cambridge-JES | 7/2/94 | W-9mx10m | No |
| Lake Shetek-Buffer | 9/9/94 | Linear-250m | No |
| Lake Shetek-High Density | 9/9/94 | Linear-250m | No |
| Lake Shetek-Low Density | 9/9/94 | Linear-250m | No |
| Lake Shetek-Residual | 9/9/94 | Linear-250m | No |
| Lake Shetek-Undisturbed | 9/9/94 | Linear-250m | No |
| Cambridge-Country Club | 9/21/94 | W-9mx10m | Yes |
| Cambridge-JES | 9/21/94 | W-9mx10m | Yes |
| MNDOT Remnant Prairie | 10/1/94 | Linear-50m | Yes |
| MNDOT Restored Prairie | 10/1/94 | W-30m wide | Yes |
| MNDOT Restored Prairie | 10/1/94 | Linear-50m | Yes |
| MNDOT Restored Prairie | 10/1/94 | Linear-30 m | Yes |
| Crosstown Prairie | 10/8/94 | W-60 m wide | Yes |
| Crosstown Prairie | 10/8/94 | Linear-50 m | Yes |
| St. Croix Rest Area | 10/8/94 | Linear-120m | Yes |
| St. Croix Rest Area | 10/8/94 | Linear- 120m | Yes |
| University Prairie | 10/8/94 | Linear-50m | Yes |
| University Prairie | 10/8/94 | Linear-50m | Yes |
| Shaefer Prairie | 10/12/94 | Linear-120m | No |
| Shaefer Prairie | 10/12/94 | Linear-120m | No |
| MnDOT Remnent Prairie | 10/14/94 | Linear-50m | Yes |
| MnDOT Restored Prairie | 10/14/94 | W-35m wide | Yes |
| MnDOT Restored Prairie | 10/14/94 | W-35m wide | Yes |
| MnDOT Restored Prairie | 10/14/94 | W-35m wide | Yes |
| MnDOT Restored Prairie | 10/14/94 | W-30m wide | Yes |
| MnDOT Restored Prairie | 10/14/94 | Linear-50m | Yes |
| Lake Shetek-Buffer | 10/15/94 | Linear-250m | Yes |
| Lake Shetek-High Density | 10/15/94 | Linear-250m | Yes |
| Lake Shetek-Low Density | 10/15/94 | Linear-250m | Yes |
| Lake Shetek-Residual | 10/15/94 | Linear-250m | Yes |
| Lake Shetek-Undisturbed | 10/15/94 | Linear-250m | Yes |
| Cambridge-Country Club | 10/21/94 | No collections | No |
| Cambridge-JES | 10/21/94 | No collections | Yes |
| Cambridge-Across from JES | 10/29/94 | W-10mx10m | Yes |
| Cambridge-Country Club | 10/29/94 | W-9mx10m | Yes |
| Cambridge-JES | 10/29/94 | W-9mx10m | No |
| Cambridge-Lake SE of JES | 10/29/94 | W-9mx10m | No |
| Fedder Prairie | 11/12/94 | NA | No |
| Inver Grove-Along pond | 11/14/94 | Linear-60m | Yes |
| Inver Grove-Disturbed Area | 11/14/94 | W-10mx10m | Yes |
| Inver Grove-Duck pond | 11/14/94 | W-10mx10m | Yes |
| Inver Grove-Undisturbed | 11/14/94 | W-10mx10m | Yes |
| Crosstown Prairie | 11/20/95 | NA | No |

## Appendix C. Spore isolation procedures [15]

For spore characterization:

The soil was left in the convection oven until completely dried. Then, a 10 to 25 gram sample, 10 ml of sodium metaphosphate (Calgon), and 90 ml of water were placed in a 500 ml screw top erlenmeyer flask. The flask was then shaken on a mechanical shaker (Eberback Mechanical Shaker). After shaking, it is decanted over a series of sieves with mesh sizes of 250, 90, and 38 µm mesh sizes. The sample was then resuspened in 250 ml of water and shaken and decanted again. This process was repeated at least two more times or until the water decanted was clear. The 250 µm sieve was rinsed carefully to wash the spores onto the smaller sieves below. The material on the 250 µm sieve and a couple of drops of 1% HCL were placed in a labeled petri plate, which was sealed with parafilm. The material from the two remaining sieves was placed in centrifuge tubes containing Kaolin. The spore solution in the tubes were stirred and then spun for 5 minutes. The supernatant was decanted over a 38 µm sieve and then rinsed through a vacuuum filter appartus. The spores were collected on a nitrocellulose filter, which was placed in an Analyslide container for storage.

The precipitate in the centrifuge tubes was resuspened in 20 ml of 2M sucrose and the tubes were balanced using water. The pellet was stirred and then spun for 2.5 minutes. The sucrose solution, containing spores, was decanted over the 38 µm sieve, which was then rinsed to wash the sucrose off. The material was then vacuum filtered and stored as noted in an Analyslide container. All spores were stored in the refridgerator until they were examined under the dissecting and compound microscopes.

For spore inoculum:

The procedure for spore isolation for inoculum was similar to the procedure for spore characterization. It differs from the spore characterization procedure in that Calgon was eliminated from the beginning step and 1% HCL was not added to the petri dishes containing the material from the 250 µm sieve.

**Appendix D. Locations of sites throughout Minnesota.**

| Site | Location |
|---|---|
| Cambridge-JES wetland | Intersection of Highway 65 and Main Street north of Cambridge, MN |
| Cambridge-Country Club wetland | Off of Highway 65 at the next intersection north of the JES site |
| Cambridge-Pond SE of JES | Highway 65 north of Cambridge, about 1/2 south of JES |
| Lake Shetek | Highway 59 at bridge 6174 |
| Mn/DOT | Just off of Highway 36, near the intersection of English and Gervais |
| Crosstown | Near the intersection of Highway 55 and Highway 62 |
| St. Croix | St. Croix rest area on the north side of Interstate 94 |
| University of Minnesota prairie | On the north side of Folwell Ave., between Gortner and Cleveland Ave. |
| Schaefer | Seven miles west of Glencoe on Highway 212, and south on a gravel road |
| Feder | Off of County road 22, near highway 15 |
| Inver Grove Heights | Near the intersection of 80th St. and Hwy 103 |

**Appendix E. Hoagland's nutrient solution that was used to water AM inoculated plants.**

**Ten-percent Hoagland's Solution**

| | | |
|---|---|---|
| $KNO_3$ | 5 ml | |
| $Ca(NO_3)_2$ | 5 ml | |
| $MgSO_4$ | 2 ml | |
| Micro. | 1 ml | **10 Liters** of double distilled $H_2O$ |
| FeNaEDTA | 1 ml | |
| $KH_2PO_4$ | 1 ml | |

**Hoagland's Nutrient Solution** [39]

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.

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/10L | mg/ml of full strength | PPM |
|---|---|---|---|---|---|---|---|
| $KNO_3$ | Sigma P6162 | 101.11 | 50.55g/500ml | 1 M | 5 | K: 1.955<br>$NO_3$: 3.100 | 1955<br>3100 |
| $Ca(NO_3)_2$-$4H_2O$ | Fisher C-109 | 236.15 | 236.15/1L | 1 M | 5 | Ca: 2.004<br>$NO_3$: 6.200 | 2004<br>6200 |
| $MgSO_4$-$7H_2O$ | | 246.48 | 123.24g/500ml | 1 M | 2 | Mg: 0.0486<br>$SO_4$: 0.192 | 48.6<br>192 |
| EDTA-$2.5H_2O$ | Sigma-EDFS | 412.04 | 3.9450g/500ml | 19.1mM | 1 | Fe: ? | |
| 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<br>Cl: 0.645 | 0.50<br>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. $(NH_4)_6Mo_7O_4H_2O$ | 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<br>$PO_4$: 0.031 | 39.1<br>31.0 |

## Appendix F. Soil coring procedures

For single spore inoculum:

Weighing dishes were labeled with the colors of tape, corresponding to the spore colors, and the sample number. Two cores were taken on opposite sides of each pot. Between a 1 or 2 g subsample was removed, weighed, and placed in the convection oven for drying. The rest of the sample was then weighted and rinsed over the 250 and 38 µm stacked sieves. Roots are picked off of the 250 µm sieve using a tweezers and placed in a labeled vial with 50% ethanol. Staining was done [11] and percent colonization was determined [49].

If needed immediately for spore counts, the soil on the 38 µm sieve was isolated according to the procedure found in Appendix C.

For general inoculum:

The procedure for soil coring for general inoculum was similar to the procedure for single spore inoculum with the following exceptions. The weighing dishes were labeled with the sample number and indicated if it was a control and not with tape colors to indicate spore types. Also, the two cores were taken from seven randomly chosen experimental pots and three of the five controls.