Final Report 1998-15

Roadside Prairie and Wetland Restoration: Mycorrhizal/Plant Factors

Technical Report Documentation Page

1. Report No.	2.	3.
MN/RC - 1998/15		PB99-109704

4. Title and Subtitle	5. Report Date
ROADSIDE PRAIRIE AND WETLAND RESTORATION: MYCORRHIZAL/PLANT FACTORS	May 1998
	6.

7. Author(s)	8. Performing Organization Report No.
Iris Charvat, Hamdy Agwa, Michael Smith, Joel Tallaksen, Jennifer White, Liza Gould	

9. Performing Organization Name and Address	10. Project/Task/Work Unit No.
University of Minnesota - Department of Plant Biology 220 Biological Sciences Center 1445 Gortner Avenue St. Paul, MN 55108	11. Contract (C) or Grant (G) No. (C) 74584 TOC #5

12. Sponsoring Organization Name and Address	13. Type of Report and Period Covered
Minnesota Department of Transportation 395 John Ireland Boulevard Mail Stop 330 St. Paul, Minnesota 55155	Final Report - 1996 to 1997
	14. Sponsoring Agency Code

15. Supplementary Notes

16. Abstract (Limit: 200 words)

In this project, researchers studied mycorrhizal and vegetational characteristics at prairie and wetland restoration areas. Study objectives included the following:

- quantifying the effect of fungal inoculum on plant communities at a Minnesota Department of Transportation (Mn/DOT) prairie restoration site near Cambridge, Minn.
- evaluating the prairie forb germination rates
- monitoring revegetation at prairie and wetland restoration sites
- characterizing mycorrhizal status of native wetland and prairie areas for comparison to the restored sites
- producing fungal inoculum for incorporation into further reclamation areas.

Findings indicated that 15 months after planting, fungal inoculation resulted in significantly greater cover by native plant species than seen in control plots. At this site, mycorrhizal inoculation benefited the prairie restoration effort by encouraging earlier, more extensive establishment of the planted species. Ongoing studies at this site will determine the long-term effects of mycorrhizal inoculation on the plant community.

The report also presents specific recommendations for future restoration efforts. The studies of mycorrhizae in native prairies and wetlands provide further data for a baseline against which to compare restored areas. In addition, fungal inoculum produced in this project has been incorporated into restoration plots at another Mn/DOT site.

17. Document Analysis/Descriptors	18. Availability Statement
Arbuscular mycorrhizae (AM fungi) fungal inoculum wetland and prairie restoration	No restrictions. Document available from: National Technical Information Services, Springfield, Virginia 22161

19. Security Class (this report)	20. Security Class (this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	66	

ROADSIDE PRAIRIE AND WETLAND RESTORATION: MYCORRHIZAL/PLANT FACTORS

Final Report

Prepared by:
Iris Charvat, Ph.D.
Michael Smith, M.S.
Jennifer White, M.S.
Hamdy Agwa, Ph.D.
Joel Tallaksen, B.S.
Liza Gould, B.S.

Department of Plant Biology
University of Minnesota
220 Biological Sciences Center
1445 Gortner Avenue
St. Paul, MN 55108

May 1998

Published by:
Minnesota Department of Transportation
Office of Research Services
First Floor
395 John Ireland Boulevard, MS 330
St. Paul, MN 55155

ACKNOWLEDGMENTS

The authors would like to thank Robert Jacobson, Leo Holm and Dr. Lawrence Foote for providing excellent assistance and support from Mn/DOT. We would also like to acknowledge all of the students, interns and technicians who worked on this research project.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

EXECUTIVE SUMMARY

The Minnesota Department of Transportation (Mn/DOT) has participated in a number of post-construction roadside reclamation projects. Such roadside areas have intrinsically inhospitable conditions, receiving higher levels of salt and lead pollution than natural areas, and making establishment of native communities difficult. The addition of native mycorrhizae to such reclamation sites may somewhat ameliorate these harsh conditions.

Arbuscular mycorrhizal fungi (AM fungi) are symbiotic fungi that form mutually beneficial relationships with higher plants. In return for carbohydrates, the fungus provides the plant with nutrients, and may increase the plant's tolerance to stressful environmental conditions. AM fungi play an important role in prairie ecosystems, where many plant species cannot establish without their mycorrhzal symbiont. In wetland ecosystems, research has shown mixed results regarding the importance of mycorrhizal fungi.

The primary goal of this project is to study mycorrhizal and vegetational characteristics at both prairie and wetland areas. Our objectives included 1) quantifying the effect of fungal inoculum on plant communities at a Mn/DOT prairie restoration site near Cambridge, MN, 2) evaluating prairie forb germination rates, 3) monitoring revegetation at prairie and wetland restoration sites, 4) characterizing mycorrhizal status of native wetland and prairie areas for comparison to the restored sites, and 5) producing fungal inoculum for incorporation into further reclamation areas.

Experimental inoculation of prairie restoration plots resulted in significantly greater cover by native plant species 15 months after seeding than seen in control plots. This result suggests that mycorrhizal inoculation was beneficial to the prairie restoration effort at this site by encouraging earlier, more extensive establishment of the planted species. Ongoing studies at this site will determine the longer-term effects of mycorrhizal inoculation on the plant community. Additionally, a similar project has been initiated at a different restoration site to determine whether the beneficial effects of mycorrhizal inoculum are repeatable at different restoration locations.

Forb germination studies show high variability among species with regard to germination rate, suggesting that germination tests before planting may be a worthwhile investment. Sand germination, while rapid and inexpensive, did not yield comparable results to soil germination for all species, making field value of this type of test questionable. Viability stains, on the other hand, accurately identified species with poor germination and are recommended before large scale seed investment.

Fifteen months after seeding in the upland prairie plots, native/desirable species comprised a majority of the vegetation, particularly in plots inoculated with AM fungi. Only three out of 21 native forb species were represented, and those at low density, indicating that a

greater percentage of forb seed may be needed in the seed mix to result in significant forb representation. In the wetland area, much of the vegetation was also comprised of native/desirable species, although reed canary grass, an exotic invasive species, was also present. Most of the species present in the wetland were also present in undisturbed adjacent wetlands, suggesting natural dissemination of propagules from these areas. Few of the species were represented in the seedbank. In future, wetland restoration efforts, areas isolated from undisturbed wetlands will require seeding, planting, or other additional effort to ensure successful establishment of native species.

Studies of the mycorrhizal status of three wetland plant species indicate that plants in drier locales have greater mycorrhizal colonization than plants under greater saturation. These results support the hypothesis that more water will result in lower levels of mycorrhizae. Mycorrhizal colonization was not found to be directly related to phosphorus and nitrogen availability under these conditions.

When compared to native, undisturbed prairies, spore density at the restored JES site was low, but this is an unsurprising result only six months after inoculation. Six additional mycorrhizal species have been identified from the native Crosstown prairie. Mycorrhizal species diversity at JES will be characterized in a future study, for comparison to Crosstown and other native prairies.

Inoculum production experiments showed that spore production was greater when soil inoculum, versus liquid spore inoculum, was used. The spores produced in this experiment were applied as soil inoculum to a restoration site at the Mn/DOT Shakopee research facility.

Chapter 1. Introduction

The Minnesota Department of Transportation (Mn/DOT) has participated in a number of post-construction roadside reclamation projects. Such projects provide an attractive roadway environment, and may support a greater diversity of flora and fauna than similar areas colonized by non-native plants such as brome grass. However, roadside areas have intrinsically inhospitable conditions, receiving higher levels of salt and lead pollution than natural areas, and making establishment of native communities difficult. The addition of native mycorrhizae to such reclamation sites may somewhat ameliorate these harsh conditions.

Arbuscular mycorrhizal fungi (AM fungi) are obligate symbionts of higher plants, increasing uptake of nutrients and water in exchange for carbohydrates [1]. These benefits can be especially important in prairie restoration projects [2], which are highly disturbed and where plants initially have little or no access to mycorrhizal fungi [3]. Previous work has shown that Mn/DOT's JES prairie restoration site near Cambridge, MN exhibits such characteristics [4]. Furthermore, it has been documented that the presence of AM fungi can affect plant community structure and competition, inhibiting the germination and growth of weedy, non-mycotrophic plant species [3, 5-7]. Weedy species, such as mustards, were prevalent at the JES site prior to restoration efforts.

The importance of AM fungi in wetland areas has been a debated topic. For years AM fungi were believed to be of minor importance in wetland systems, but more recently this view has been challenged. For example, Sengupta and Chaudhuri [8] reported that all of the primary plant colonizers in a saline wetland showed AMF root colonization levels of 60-70%. Søndergåard and Laegåard [9] speculated that AMF may influence nutrient uptake of plants in nutrient poor, sandy, wetland soils. Pond *et al.* [10] and Stenlund and Charvat [11] have found AM fungi present in cattail (*Typha* spp.) but did not find arbuscules. Pond *et al.* [10] suggested that the absence of arbuscules might indicate that AM fungi are parasitic under these circumstances. Clearly the role of AM fungi in wetlands is far from being resolved, much less their role in wetland restoration areas.

The JES restoration site, being composed of both upland prairie and wetland areas, represents a valuable opportunity for understanding mycorrhizae at restoration sites under different ecological conditions, as well as monitor other vegetational characteristics in restoration areas. Our specific objectives in this project were to:

1) test the effect of mycorrhizal amendments at a prairie restoration site,
2) test the germination and viability of prairie forb seeds,
3) monitor revegetation at upland prairie and wetland restoration areas,
4) monitor mycorrhizal parameters at undisturbed wetland and prairie areas for comparison to restoration areas, and
5) investigate spore production for use as inoculum at restoration sites.

Chapter 2 is a study of the effects of mycorrhizal amendments at the JES Mn/DOT restoration site, and addresses objective #1. This chapter also provides guidelines for future testing of mycorrhizal amendments at other restoration sites. Chapter 3 addresses objective #2 by comparing germination of five prairie forb species on different media, and testing seed viability.

Objective #3 is covered by Chapter 4, which discusses vegetational composition of JES upland and wetland areas, as well as potential propagule sources for the observed vegetation.

Objective #4 is addressed by both Chapters 5 and 6. Chapter 5 describes the mycorrhizal condition of three wetland plant species under different saturation levels at undisturbed wetland sites at Cedar Creek Natural History Area. Chapter 6 addresses mycorrhizal diversity for the upland areas of the same site, for contrast with previously documented mycorrhizal diversity at undisturbed prairies and with mycorrhizal diversity at the JES restoration site.

Finally, Chapter 7 addresses objective #5 by describing an inoculum production experiment. The inoculum produced by this experiment has already been incorporated into an ongoing Mn/DOT restoration study at the Shakopee experimental complex.

The research presented in this report represents an important step towards understanding the dynamics of mycorrhizal and vegetational interactions at prairie and wetland sites. Moreover, by characterizing the establishment and effects of AM fungi at a Mn/DOT restoration site, and monitoring revegetation at this site, this report has specific management implications which will be discussed on a chapter by chapter basis.

Chapter 2 The significance of arbuscular mycorrhizae in an early successional tallgrass prairie reclamation

2.1 Overview

Arbuscular mycorrhizal fungi (AM fungi) can benefit plants by increasing the uptake of nutrients and water, thereby increasing the health and vigor of the plant host [12]. These benefits can be especially important in prairie restoration projects because a number of prairie grass species are obligately mycorrhizal [2]. Furthermore, AM fungi can also affect plant community structure and competition [3, 5, 7]. It has been documented that the presence of AM fungi can inhibit the germination and growth of weedy, non-mycotrophic plant species, increase plant diversity [13], and increase the competitive effects among native prairie species [6]. These effects of the plant community are pronounced at sites that are highly disturbed where plants have little or no access to mycorrhizal fungi [3].

These facts suggest that mycorrhizal inoculation may be beneficial in prairie restoration efforts. However, the effectiveness of mycorrhizal inoculation depends on a variety of factors, including the fungal composition of the inoculum, the species composition of the plant community, and environmental conditions such as soil nutrient content and pH [14, 15]. AM fungal species have been shown to respond individualistically to cropping history and edaphic factors [16]. Under some conditions, some AM species have been shown to be less efficient mutualists or even act as parasites on their host plants [17, 18]. Coadaptation of host and endophyte populations may also occur [19].

Consequently, it cannot simply be assumed that mycorrhizal inoculation will have a positive effect on the plant community, and at this time the benefits of mycorrhizal amendments need to be tested on a site by site basis. This chapter has two major goals: first, to provide guidelines for experimenters to perform small scale tests of mycorrhizal amendments, and second, to discuss results of this type of test at a specific restoration site, namely Mn/DOT's JES restoration site.

2.2 Generating inoculum

2.2.1 The inoculum source

The first step in performing an inoculation experiment is to obtain a mycorrhizal inoculum. Although commercial inoculum is available, none is available from Minnesota, and concerns about introduction of non-native mycorrhizal species lead us to produce our own inoculum from a native prairie source. By doing so, we hoped to generate a high diversity fungal inoculum which was as close to the native fungal community composition as possible.

Our inoculum source was Crosstown prairie, which is a remnant prairie in the Twin Cities metropolitan area. Two major factors encouraged use of this site. First, Crosstown prairie is less that 80 kilometers from Cambridge, the site where the inoculum would eventually be used, thus minimizing the geographic distance between the two prairies, and increasing the chances for compatible plant/fungal interactions. Second, since the prairie restoration site is a post construction area near a major highway, the community is likely to be subject to a relatively high level of pollution, including lead and salt. Crosstown prairie is also near a roadside, has already been subject to high pollution conditions, and is likely to contain fungal species tolerant to these conditions.

Because Crosstown prairie is located on land maintained by Mn/DOT, permission to remove soil was obtained directly from our Mn/DOT liaison. However, to minimize our disturbance of the prairie, soil samples were taken from an area just outside the protected prairie which was still dominated by prairie vegetation. All soil samples were taken from soil directly under prairie plant species.

2.2.2 Pot culture method of inoculum generation

To increase our amount of inoculum, a trap-culture procedure was used. This procedure was described in more detail in a previous Mn/DOT report [4], as well as chapter 7 of this publication. A trap culture can generate a diverse mycorrhizal inoculum, containing multiple fungal species. A 1 cm^3 layer of prairie soil was placed between layers of steam sterilized greenhouse soil. Three little

bluestem seedlings (*Schizachyrium scoparium* (Michx.) Nash.) were planted in each 5.5 inch pot. Little bluestem was used because it is a native prairie grass, and previous studies [4] show relatively high spore production in trap culture using this host species. Moreover, little bluestem is one of the seeded species used at the restoration site.

After transplanting, pots were maintained in a growth chamber with a 12: 12 photoperiod and thermoperiod (25 °C: 20 °C) and watered with 50 ml 10% Hoagland's solution once a week and with tap water when needed. After one month, pots were transferred to the greenhouse and watered once a week with 100 ml 10% Hoagland's solution and with tap water daily. Low nutrient concentrations were used to encourage formation of mycorrhizal relationships. Plants were grown for a total of 19 weeks at which point watering was stopped. Aboveground plant mass was clipped and the dried soil was refrigerated for one month until needed.

2.3 Study site

The JES study site is located in Isanti County Minnesota, near the city of Cambridge, 56 km north of St. Paul, MN (45° 60′ N, 93° 21′ W). This area is located on the Anoka Sand Plain, which consists of Anoka fine loamy sands: deep glacial outwash sands that are somewhat excessively drained. Annual precipitation and temperature average 71 cm and 18.5 °C, respectively. The site was graded in 1994 as part of a road construction project. Top soil was stockpiled under unknown conditions and spread over the site after construction. A cover crop of annual rye (*Lolium perenne* var. aristatum), oats (*Avena sativa*) and Re-green (sterile hybrid of *Triticum aestivum* and *Elymus trachycaulus* (HybriTech Inc., Wichita Kansas)) was planted at that time to stabilize the soils. The restoration area consists of both wetland and upland prairie ecotypes (Fig. 2.1).

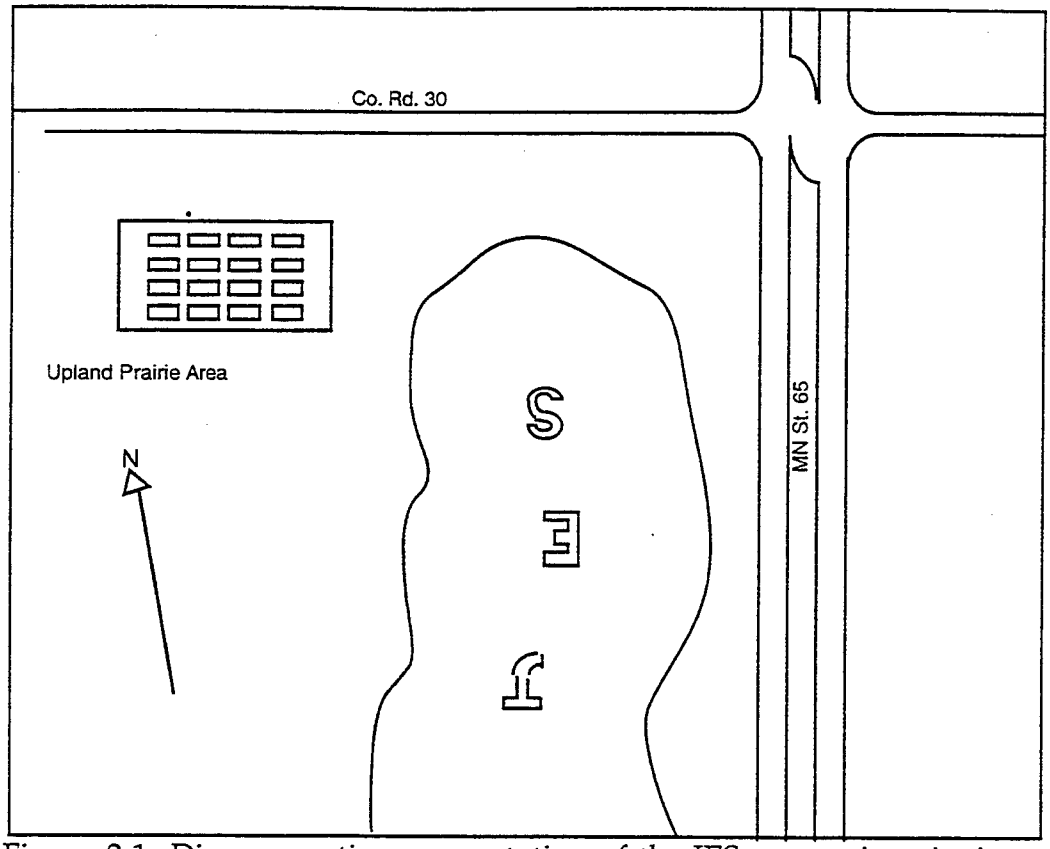

Figure 2.1 Diagrammatic representation of the JES restoration site in Cambridge, MN.

In June 1995 soil cores were taken randomly throughout the study site, pooled and analyzed to determine background nutrient levels of the area before restoration efforts began. The results from these tests are presented in Table 2.1. The soil contained a high concentration of phosphorus, but low nitrogen. Low nitrogen values indicate that there was little organic material present. The pH was reasonable for seed and fungal spore germination, and should not have inhibited symbiosis establishment. However, the phosphorus levels found at the site were high, which would not tend to encourage mycorrhizal colonization.

Table 2.1 Soil characteristics and mycorrhizal condition of the JES site prior to restoration efforts.

Soil	Soil nutrient characteristics			Mycorrhizal
type	Bray-P	NO_3-N	pH	Colonization
Fine loamy sand	69 ppm	1.3 ppm	6.4	0.4 ±0.2 %

Root material collected from these soil cores was used to estimate total background AM root colonization levels for the site (see section 2.5.1 for percent colonization methodology). We found that mycorrhizal colonization was extremely low, averaging less that one half of 1 percent of roots colonized (Table 2.1).

2.4 Testing the effect of mycorrhizal inoculum - setting up the experiment

2.4.1 Experimental design

To look at the effect of mycorrhizal inoculation on prairie restorations, 24 1 X 2 m plots were set up and given one of three treatments: native prairie seed + mycorrhizal inoculum, native prairie seed + sterile soil (control), or only native prairie seed (control) (hereafter referred to as the inoculated treatment, uninoculated soil control treatment and uninoculated control treatment, respectively). The soil control treatment was originally designed to test for alternative effects of inoculum addition, aside from the mycorrhizae itself. For example, the inoculum provides a limited pool of available nutrients to plants in the inoculated plots which are unavailable to plants in the uninoculated plots. An ideal control would consist of sterilized mycorrhizal inoculum, such that all characteristics (nutrient availability, etc.) would be constant between the inoculated treatment and the soil control treatment. However, because our supply of inoculum was limited and we did not want to waste some by sterilizing it, we used sterilized greenhouse soil instead. The greenhouse soil was not ideal, because the nutrient characteristics of inoculum versus sterilized soil were not identical (Table 2.2). The inoculum and sterile soil had comparable levels of extractable phosphorus, but the sterile soil had much greater levels of NO_3 than the inoculum. However, the soil control treatment is still useful, because it still tests the effect of adding a limited pool of nutrients without adding mycorrhizae.

Table 2.2. Soil characteristics for the inoculum and the sterile soil.

Soil Origin	Soil Type	Bray-P	NO_3-N	pH
Inoculum	Loam	101 ppm	0.08 ppm	6.9
Sterile Soil	Loam	128 ppm	198 ppm	NA

Twelve out of the 24 plots were used to measure below-ground parameters and twelve were used to measure above-ground parameters, resulting in 4 replicate plots for each treatment. This was done to minimize the effects of sampling on the vegetation, so that long-term monitoring of the plots could be maintained. A completely randomized design was used, such that each plot was randomly assigned to treatment type and measurement type (above-ground or below-ground).

2.4.2 The seed mix

All plots received approximately 2.84 g of prairie seed (27 lbs/acre) in a 25:1 grass:forb mix (Table 2.3). All seed was obtained from Prairie Restorations Inc. located in Princeton, MN approximately 29 km from the study site.

2.4.3 The inoculum

The soil and roots from the little bluestem trap culture were used as inoculum for the study. This sort of "whole inoculum" contains mycorrhizal spores in the soil, and other fungal structures (arbuscules, hyphae) in the root fragments which are capable of vegetative colonization of new plant material. Previous studies [4] have shown that whole inoculum can be more effective than spore-only inoculum. The inoculum was sieved through 1 cm hardware cloth, roots were cut to 1 cm pieces and all of the pots were homogenized. The inoculum was analyzed for spore density using density centrifugation [20, 21], and found to have 38 spores/gram of soil. Sterilized soil used as a control was treated with a microbial rinse obtained from three pots of inoculum. This was done to make the sterilized soil resemble the inoculum as closely as possible.

Table 2.3. List of species and percentages in seed mix used at the Cambridge, MN prairie restoration site, June 1995.

Plant species	common name	Percentage in seed mix*
Andropogon gerardii	big bluestem	35%
Schizachyrium scoparium	little bluestem	24%
Sorghastrum nutans	Indian grass	24%
Bouteloua curtipendula	side-oats grama	8.6%
Elymus canadensis	Canada wild rye	2.4%
Panicum virgatum	switch grass	2.4%
Rudbeckia hirta	black-eyed susan	0.50%#
Dalea purpurea	purple prairie clover	0.50%
Heliopsis helianthoides	common-ox-eye	0.32%
Monarda fistulosa	wild bergamot	0.22%
Amorpha canescens	leadplant	0.22%
Dalea candida	white prairie clover	0.18%
Asclepias tuberosa	butterfly weed	0.18%
Aster oolentangiensis	azure aster	0.18%
Zizia aurea	golden alexanders	0.14%
Liatris pycnostachya	tall blazingstar	0.14%
Aster laevis	smooth aster	0.14%
Verbena stricta	hoary vervain	0.14%
Agastache foeniculum	giant hyssop	0.14%
Lespedeza capitata	bush clover	0.11%
Solidago rigida	stiff goldenrod	0.11%
Solidago speciosa	showy goldenrod	0.11%
Solidago nemoralis	gray goldenrod	0.07%
Solidago ptarmicoides	upland goldenrod	0.07%
Coreopsis palmata	stiff tickseed	0.04%
Helianthus rigidus	stiff sunflower	0.04%
Achillea millifolium	common yarrow	0.04%

* Percentages for grasses are percentage pure live seed. Percentages for forbs are by bulk weight.

Forb species represent Prairie Restoration's "mesic wildflower mix."

2.4.4 The plots

The 24 1m x 2m experimental plots were established in mid-June, 1995 over a total area of 364 m^2. A buffer zone of two meters was placed between each plot. Within each plot, 5 furrows were made at 20 cm spacing. Furrows were made using a belt seeder (custom made by M. Moore, Dept. Plant Pathology, University of Minnesota). In order to get even distribution in the furrows, inoculum, sterile soil and seed were spread evenly in a 2 meter long 4" PVC pipe which was cut in half before they were placed into the furrows. 350 g of inoculum or sterile soil (approximately 875 g/m^2) were placed in each furrow, seed was placed on top and the furrow was then covered with soil from the site. In all cases, furrows were dug so that final seed depth was 0.25 inch. Inoculum, sterile soil and seed were all weighed out prior to placing in the field in order to deliver consistent amounts among furrows and plots.

2.5 Evaluating inoculum effectiveness

In order to determine whether mycorrhizal inoculation is useful at a particular restoration site, two factors need to be assessed. First: does inoculation result in significantly greater mycorrhizal levels than non-inoculated treatments? (In other words, does the mycorrhizal inoculation work?) This can be assessed in one of three ways - either samples of soil can be collected, and spores can be isolated by density centrifugation [20, 21], or root samples can be collected and percent fungal colonization of the roots can be evaluated [22], or the hyphal network can be measured [23]. All three methods provide a general estimate of mycorrhizal presence. However, only the root samples show that a functional, mycorrhizal relationship is in effect between fungus and plant. Consequently, we chose to evaluated mycorrhizal presence using the magnified intersect method of determining percent root colonization (see below). While somewhat time-consuming, this method allows quantification of arbuscules, and confirms mycorrhizal symbiosis. An older method of determining percent colonization, the gridline intersect method [22] is a more rapid way to determine percent root colonization, but arbuscules cannot be seen because it uses a lower magnification.

Once the effectiveness of mycorrhizal inoculation is established, the next step is to determine whether increased mycorrhizal colonization corresponds to a beneficial effect for the plants and/or the plant community. Many measures could be used to evaluate this, including overall plant biomass, plant reproductive activity, or plant community composition. For this study, the specific question of interest is whether mycorrhizal inoculation significantly helps in the establishment of planted native species, as opposed to non-native ruderal or weedy species. Consequently, we have used percent cover of native planted versus unplanted weedy species to evaluate mycorrhizal effect. Our hypothesis is that mycorrhizae provide greater benefits for native planted species, which are often obligately mycorrhizal, than for non-native weedy species which often do not form mycorrhizal relationships. If this is true, then over time, native species should show a greater response to the mycorrhizal inoculum, and attain greater percent cover when inoculated than when not. Non-native species would not be as likely to show this response.

2.5.1 Mycorrhizal colonization

In September 1996 (15 months after seeding) soil cores were taken directly adjacent to the furrows to a depth of 5 cm using a 3/4" diameter soil probe. Five cores were taken at random locations along each furrow resulting in 25 cores per plot. These 25 cores were thoroughly homogenized and used for root isolation. Roots were isolated over a 250 µm sieve, washed free of debris and preserved in 50% ethanol. Clearing and staining procedures followed methods modified from Phillips and Hayman [24], Kormanik et al. [25], and Koske and Gemma [26]. All roots obtained from the site were cleared overnight in 10% KOH, acidified for one hour in 1% HCl, stained overnight with 0.05% trypan blue and destained with acidic glycerol. A randomly selected subsample of the stained roots from each plot was mounted on microscope slides. Percentage AM colonization was determined using the magnified intersection method [27]. Roots were examined at 160X and 400X. Four replicates of 100 intersections each were counted for each plot. Total mycorrhizal colonization was calculated by adding vesicular, arbuscular and AM

hyphal colonization. AM hyphae were distinguished from hyphae of unknown origin by the presence of mycorrhizal structures attached to them. If the eyepiece crosshair intersected a hypha that was attached to a vesicle or arbuscule within the field of view, this hypha was counted as an AM hypha.

2.5.2 Plant response

Percent cover was measured 3 months after seeding (September 1995) and 15 months after seeding (September 1996) using the point frame method [28]. The wooden frame was constructed to fit over the one meter wide plots. Ten flags were evenly spaced along this one meter distance on the frame. The entire frame was placed ten times in each plot at 20 cm intervals yielding 100 points per plot. This ensured that percent cover estimates were taken over the entire plot. Each time an individual leaf, flower or stem touched the flag, its presence was recorded, even if it was from the same individual. Because multiple vegetation contacts could be made at a single point, percent cover could well exceed 100%. If the plant touching the flag was dead this was recorded next to the species code. If the plant could not be identified, it was either collected from outside the plot for future identification or recorded as an unknown. The senesced basal rosette of leaves often present at the base of warm season prairie grasses were recorded, but were not recorded as dead because they are part of a living plant. Many of the warm season native grasses were too immature to identify to species and were placed in their own general category. This category consisted of *Andropogon gerardii* (big bluestem), *Panicum virgatum* (switch grass) and *Sorghastrum nutans* (Indian grass). If one of these plants could be identified to species (i.e. if it were found flowering), it was recorded in its own category. For statistical analysis, however, the separate categories were placed into one group. All other native grasses could be identified to species.

2.6 Evaluating mycorrhizal inoculum - results and discussion

2.6.1 Mycorrhizal colonization

Mycorrhizal colonization of plants in 1996 (mean ± SEM) was 44.2% ± 1.9 in the inoculated treatment, 35.3% ± 1.7 in the uninoculated soil control treatment and 37.7% ± 1.4 in the uninoculated control treatment (Figure 2.2). The inoculated treatment had significantly greater mycorrhizal colonization than either control treatment ($p=0.012$).

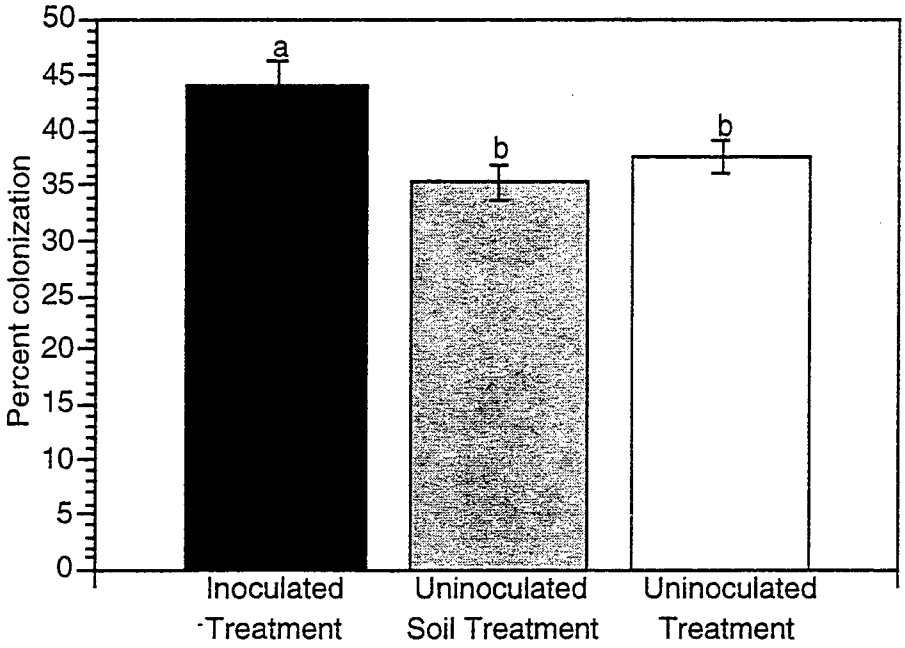

Figure 2.2 Mycorrhizal colonization in inoculated, uninoculated soil, and uninoculated treatments, 15 months after experimental set-up. Columns with the same letter did not differ significantly at $\alpha = 0.05$.

Although the treatments differed significantly in percent colonization, the absolute differences in values were not great, differing at most by 10%. Even the uninoculated treatments had 35% colonization after 15 months. This is probably due to natural colonization from the surrounding area by windborne spores. The inoculated treatments likely received a jump start in mycorrhizal colonization, and achieved higher levels of colonization faster than the uninoculated treatments.

However, natural colonization of the uninoculated treatments would cause percent colonization in these treatments to gradually increase, eroding the effect of mycorrhizal inoculation. Had percent colonization been evaluated earlier, it is likely that the differences among treatments might have been greater. Regardless of the magnitude of the difference among the treatments, however, the fact remains that inoculation of plots resulted in greater levels of mycorrhizal colonization than in uninoculated plots.

2.6.2 Plant response

Three months after planting (September 1995), total vegetation cover did not differ significantly among treatments (Figure 2.3). All of the treatments were dominated by unplanted weedy species, including horseweed (*Conyza canadensis*), Kentucky bluegrass (*Poa pratensis*), crab grass (*Digitaria ischaemum*), foxtail (*Setaria* sp.), common plantain (*Plantago major*), white campion (*Silene latifolia*),and ragweed (*Ambrosia artemisiifolia*). The cover crop species also made a sizable contribution to percent cover in all treatments (Figure 2.3). Very few of the native planted species had germinated at this time. Percent cover of these three groups (native planted, weedy unplanted, and cover crop) did not differ significantly among treatments.

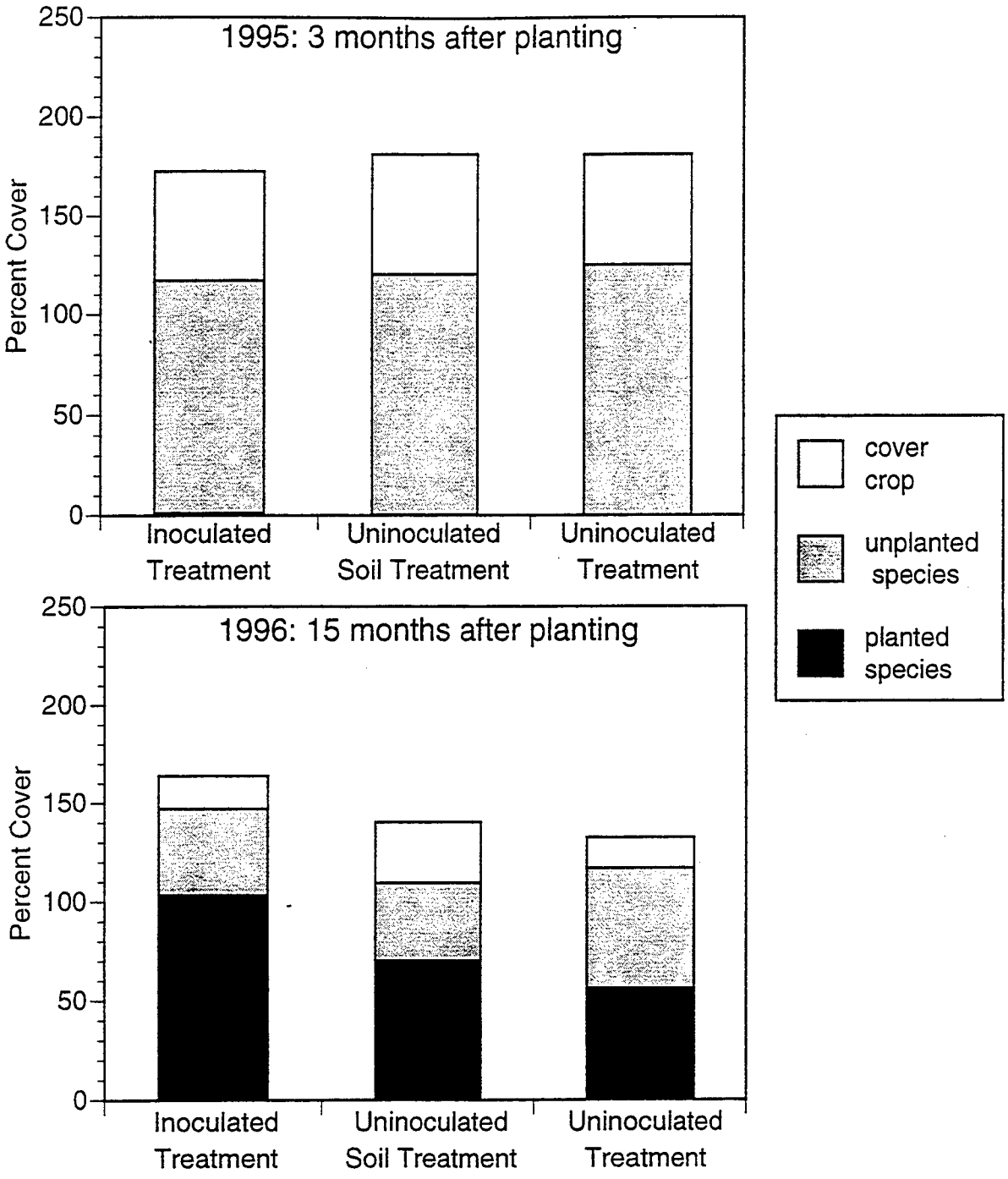

Figure 2.3. Percent cover of native seeded species, unplanted weedy species, and cover crop 3 months and 15 months after seeding. At 15 months, inoculated plots had significantly greater cover of native seeded species than either uninoculated treatment. Total percent cover, percent cover of weedy species, and percent cover of cover crop species did not differ significantly among treatments at any time point. For all treatments, cover of native planted species greatly increased over a 1 year period, while unplanted species and cover crop species declined.

Total vegetation cover after 15 months (September 1996) was somewhat lower than it had been at 3 months (Figure 2.3), and once again did not differ among treatments. The plant community had shifted dramatically between years. In contrast to 1995, the 1996 data showed that planted native species made up the greatest component of the cover in all treatments, followed by unplanted weedy species, while cover crop species contributed only a small amount of cover to the plots (See Chapter 4 for further details regarding plant species composition).

When these data were statistically analyzed, an interesting pattern emerged. The inoculated treatment had significantly greater percent cover of native planted species than either of the control treatments. This contrasts with percent cover of unplanted weedy species and cover crop species, which did not differ significantly among treatments.

This increase in percent cover of native species in the inoculated plots can be attributed mainly to the native planted grasses, which contributed approximately 90% of the cover in the inoculated plots. The planted forbs (mainly black-eyed susan and common ox-eye) also contributed a small amount of cover (3%), although the differences were not significant among treatments.

Mycorrhizal inoculation resulted in increased percent cover of native planted species, but did not have an effect on percent cover of unplanted weedy species or cover crop species. This suggests that mycorrhizal inoculation at this site was beneficial to the prairie restoration effort by encouraging earlier, more extensive establishment of the planted species. Ongoing studies at this site will determine the longer-term effects of mycorrhizal inoculation on the plant community.

2.6.3 Conclusions

The results of this study suggest that it is worthwhile to incorporate mycorrhizal inoculum at future restoration sites. However, some caution must be used in interpreting these results. Plant/mycorrhizal interactions can be highly individualized, and depend on many variables (plant species composition, fungal community composition, soil + other environmental characteristics, etc.). Consequently, the results from a single study should not be extrapolated to all sites

and situations. However, using the guidelines provided in this chapter, a body of studies on the effects of mycorrhizal inoculation in restoration efforts can be developed, and from these studies, predictions about the benefits of mycorrhizal inoculation in a broad array of situations can be made.

Chapter 3 Germination and seed development studies

3.1 Overview

The restoration of native prairie plant communities has received considerable attention in recent years. Native prairie ecosystems often support a greater diversity of flora and fauna than similar areas colonized by non-native plants [29]. In an effort to lessen the impact of road construction, Mn/DOT has undertaken numerous prairie restoration projects throughout the state. At the JES study site described in chapter 2, Mn/DOT has seeded a native prairie mix into disturbed roadside soil. This seed mix is primarily composed of warm season grasses, with smaller components of native forb species (Table 2.3).

Prairie restoration suppliers typically publish statistics on germination rates of grass species seeds, but do not provide similar data for prairie forbs. This data could be particularly important because forb seeds are expensive and typically represent only a small portion of seed planted in prairie restoration efforts. Low percentage germination may mean these species will not be represented at all. Moreover, the seeds of some forb species (e.g., the prairie clovers, *Dalea* spp.) develop within persistent calyxes, and the calyxes provided by a seed company may or may not actually contain seed within them. This would further decrease the number of viable seeds per gram planted. This is an undesirable outcome, since some forb species (*e.g.*, the prairie clovers and other nitrogen fixing legumes) play important roles in the prairie ecosystem. Natural prairies may be composed of up to 40% forb species [29].

Consequently, it may be beneficial to test seed germination *a priori* before investing in large scale seeding efforts, especially if future restoration efforts involve larger proportions of forb seeds. The easiest method to test germination is to simply observe germination on saturated sand medium. However, these conditions do not closely mimic conditions experienced by seed in the field, and may give an inaccurate representation of field germination levels. The objective of this study is to test 5 forb species used in the standard Mn/DOT mix for germination rates under both sand and soil conditions. By comparing these values, as well as monitoring

recruitment under field conditions, we should not only gain valuable information about germination rates of these species, but also be able to make conclusions about the usefulness of sand germination in predicting field performance.

3.2 Materials and methods

3.2.1 Comparison of percent germination of dominant prairie forb species under controlled conditions

Two germination experiments were conducted using five forb species:, *Aster oolentangiensis* (azure aster), *Heliopsis helianthoides* (common ox-eye), *Dalea candidum* (white prairie clover), *Dalea purpureum* (purple prairie clover), and *Rudbeckia hirta* (black-eyed susan). Germination on wet sand in petri dishes was compared with germination in greenhouse soil. For the sand germination experiment, 50 ml silica sand was autoclaved in clean petri dishes. Six replicates (100 seeds per replicate) were established. Seeds were sterilized by immersion in 5% bleach solution for 5 minutes and rinsed with 5 changes of distilled water. Purple prairie clover and white prairie clover were scarified as outlined in 3.2.2. Seeds were placed into Scherr growth chambers under 12 hour photoperiods and thermoperiods (30 °C: 15 °C). Seeds were monitored for germination under a dissecting scope at 12X magnification daily until day 11, after which dishes were monitored on alternating days until day 29. Water was added to the petri dishes after each observation. Germination was defined as appearance of the radicle (the embryonic root).

A similar germination study was conducted in soil, with 100 seeds of each forb species planted in greenhouse flats containing sterilized greenhouse soil and 1:1 perlite:vermiculite mix. Flats were placed in growth chambers under the same conditions as described above. Seeds in this experiment were also monitored for germination until day 29. Germination was defined as appearance of a shoot at soil surface. The percent germination in soil was compared to sand germination using chi-squared contingency table analysis.

3.2.2 Seed development in white and purple prairie clovers

Both white and purple prairie clovers have seeds that develop within persistent calyxes. The calyx is often hairy and conceals the seed. In order to determine the percentage of calyxes which actually contain seeds, six replicates of 100 seeds each were scarified. Scarification was done between two pieces of sand paper in such a way that the calyx is removed and the seed inside, if any, becomes visible. Note that only one seed may develop within each calyx.

3.3 Results and Discussion

3.3.1 Percent germination

Figure 3.1 shows the results for the germination of seeds on sand and soil. Average percent germination on sand was found to be approximately 2% in azure aster, 68% in common ox-eye, 69% in white prairie clover, 55% purple prairie clover, and 25% in black-eyed susan. In some cases, the results were similar when germinated in greenhouse soil. Percent germination in azure aster and the two prairie clovers did not differ significantly between sand and soil at $\alpha = 0.05$. This illustrates that defining germination as appearance of the radicle for seeds on wet sand provided an accurate prediction for germination success in soil for these three species.

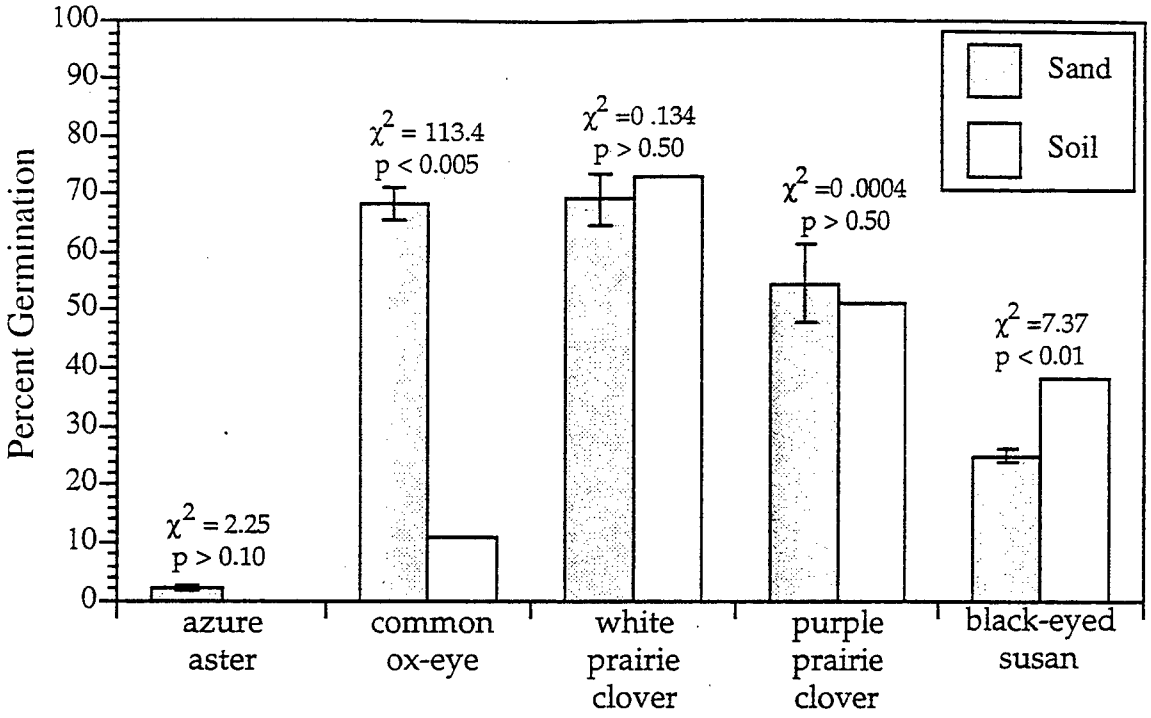

Figure 3.1 Comparison of percent germination of five prairie forbs in sand and soil after 29 days in a growth chamber. Germination on different media was compared for each species using chi-square analysis at $\alpha = 0.05$.

For the other two forb species, however, germination on sand was significantly different than germination on soil at $\alpha = 0.05$. Percent germination in black-eyed susan was somewhat lower on sand than in soil, whereas common ox-eye showed nearly seven times greater germination on sand than in soil. For these species, germination on sand is a poor indicator of germination in soil.

In order to evaluate the viability of the ungerminated seeds in the sand experiment, a viability test was conducted. The remaining seeds were soaked in triphenyl tetrazolium chloride (TTC) for 24 hours and then examined. If the seed is viable, TTC will stain the endosperm red. The results from this study are show in Table 3.1.

Table 3.1 Percent viability of ungerminated seeds of three forb species determined using triphenyl tetrazolium chloride (TTC).

Species*	Number of remaining seeds	Number of viable seeds	Percent viability
Azure aster	362	9	2.5%
Common ox-eye	153	18	3.6%
Black-eyed susan	304	29	9.4%

* No remaining seeds of the prairie clovers were viable.

For azure aster and common ox-eye the viability of the remaining seeds was low enough to assume that the germination requirements for the seeds were, for the most part, met in the germination experiment. Further investigation of the azure aster seeds obtained from Prairie Restorations Inc. revealed that they were infected with a fungus and were non-viable, explaining the overall low rates of germination in this species. No azure aster plants have been found at the JES study site. Approximately 9.4% of the remaining black-eyed susan seeds were still viable. Unlike the other forb species, in which the majority of seed germinated within the first two weeks of the sand experiment, black-eyed susan seeds germinated throughout the four week experiment. This suggests that black eyed susan seeds may require a longer period to germinate, and perhaps the remaining viable seeds would have germinated in time. Alternatively, the sand experiment may not have been meeting the germination needs for this species. This may also explain the relatively low percent germination number obtained for black-eyed susan.

Data from the germination of forbs in September, 1996 at the Cambridge site are presented in Table 3.2. Only two of the planted forb species were found in relative abundance at the site: black-eyed susan and common ox-eye. (A few individuals of wild bergamot, *Monarda fistulosa* and giant hyssop, *Agastache foeniculum* were also found). Black-eyed susan was present in the surrounding area in summer of 1995 and 1996, and may have recruited naturally into the restoration area.

Neither of the two prairie clover species were found at the site. This could be the result of the low germination results presented above and in 3.3.2. It could also

be that the specific requirements for germination of these species were not met at the JES site. Some of the seeds may not have germinated because of the extreme period of hot, dry weather immediately following planting. These species and others not found this year may germinate in subsequent years; prairie clovers in particular have been shown to exhibit germination delays for a period of years (R. Jacobson, pers. obs.). It is possible that the relatively late seeding date in this study may have driven the seeds to dormancy. Monitoring of this site in the future will track the germination of all species planted and determine the success of the forb planting.

Table 3.2. Forb numbers at the Cambridge prairie restoration plots, September, 1996.

Species	Mean ± 1 SEM
Black-eyed susan	4.8 ± 1.12*
Common ox-eye	0.17 ± 0.11
White prairie clover	0
Purple prairie clover	0
Azure aster	0

* Numbers presented are averages ± standard error

3.3.2 Seed development in the prairie clovers

The results of this study are shown in Table 3.3. Many calyxes did not have seeds present within them. Only ~40% had developed seeds for both species.

Table 3.3. Seed development in the persistent calyx of prairie clovers

Species	Mean % Seed Development ± 1 standard deviation
White prairie clover	40.0 ± 4.82
Purple prairie clover	40.8 ± 9.83

Given the above information on seed viability and germination both in the lab and in the field, some recommendations can be made concerning future seedings. For the clover species, the number of seeds may need to be increased by 60% to obtain numbers comparable to other species. The black-eyed susan and common ox-eye seeds germinated very well in the field in fall of 1995 and 1996 and should remain in the seed mix at their current levels. Wild bergamot was also found in fair numbers (a total of 11 individuals in all of the plots) and should re-seed successfully. Only one individual of giant hyssop and 3 of *Achillae millifolium* (common yarrow) were found at the site. The rest of the plant species in the mix have not yet germinated. These ungerminated species make up approximately 70% of the wildflower mix by weight. Once again, this may be a result of the harsh weather conditions at the time of planting.

The result of germination on sand and soil suggest that the value of sand germination tests will be species dependent. That is, in some forb species sand germination is well correlated with soil germination, whereas other species show a poor correlation between the two values. We therefore do not recommend sand germination tests as standard practice before planting in future restoration efforts. However, small scale viability tests using TTC may well be worthwhile, as this would allow identification of species with low seed viability before planting.

Chapter 4. Plant colonization at the Cambridge site: revegetation and seed bank study

4.1 Overview

In 1994 an area north of Cambridge Minnesota was severely disturbed when the Minnesota Department of Transportation (Mn/DOT) constructed a new roadway (Mn state highway 65). After construction was completed, the area was refilled such that both wetland (JES pond) and drier upland areas were created (Figure 2.1). Both fill and the original topsoil were used, and a cover crop of annuals and short-lived perennials was planted at that time to control erosion. In 1995, portions of the upland area were seeded with a native prairie mix, as part of an experiment to determine the effects of mycorrhizal inoculation on native species establishment (Chapter 2). Also in 1995, many native wetland species were planted at plots south and east of JES pond by Dr. David Biesboer from the U of MN Plant Biology Department and Bob Jacobson, Mn/DOT Botanist.

There are many potential propagule sources which could contribute to revegetation at JES. In addition to the species planted at the site, vegetation from nearby undisturbed areas could provide propagules to the disturbed site. This is particularly true of the wetland ecotype, which is connected hydrologically to undisturbed wetland areas to the north and east. Additionally, vegetation could originate from seed in the seedbank.

The purpose of this chapter is to monitor revegetation at both the planted upland areas and the unplanted wetland areas of the JES site. The species list from JES will be compared to that of the planted species, seedbank species, and adjacent wetland species, in an effort to understand the contribution that each of these sources has made towards revegetation at the JES site.

4.2 Materials and methods

4.2.1 Seed bank status at Cambridge upland and wetland sites

Soil cores from the upland prairie experimental plots were taken along a transect on May 15, 1995 for seed bank analysis. This was done before any planting of native species had taken place. Soil cores for the wetland seed bank were taken on August 24, 1995 in a straight line transect along the north bank of the JES pond, located in close proximity to the upland site. All cores were taken from the top 5 cm of the soil only.

Soil cores from each habitat (upland, wetland) were homogenized and spread 1 cm thick on top of 3 cm of sterile greenhouse soil with a 2:1 vermiculite:perlite mix. Flats were then placed into the College of Biological Sciences Greenhouse and monitored for seedling emergence. Once plants became identifiable, they were recorded and removed from the flat.

Four replicates of the upland seedbank study were established and watered once per day. These flats were monitored once a week. Two different watering treatments were established for the wetland seedbank study. The first treatment, containing four replicate flats, was watered once per day. The second treatment, also containing four replicate flats, was maintained in a constantly saturated state. These differing treatments allowed the establishment of seeds with differing germination requirements [30].

4.2.2 Revegetation in the upland plots

Percent cover measures were taken in the upland plots in September 1995 and September 1996, using the point frame method [28] as described in chapter 2. It was noted for each species whether it was planted (member of seed list, table 2.1), found in the seed bank, or seeded as a cover crop.

4.2.3 Revegetation at JES pond

In order to determine the rate of plant re-colonization at the Cambridge wetland site and the potential for plant community development, surveys of the area were conducted throughout the growing season. The north shore and approximately northern one-fifth of the west shore of JES pond were inventoried with 5 randomly located 1m x 2m plots; 3 plots were located at the water's edge in late June when the first sampling was conducted, and the other 2 were located approximately 6 m from the June high water point on the north shore. In addition, a series of transects were laid parallel to the north shore and from the northern one-fifth of the western shore of JES pond. Less formal surveys the areas north of County Road 30 and east of Highway 65 were conducted in mid-July and late August to gain information on possible seed sources for plant recolonization at the JES wetland.

4.3 Results and Discussion

4.3.1 Seed bank studies

Table 4.1 lists all of the plant species found in the seed bank experiment for the upland JES site. Given the history of this site, it is not surprising to find that approximately 50% of the plants present in the seed bank are species that typically colonize disturbed areas. These seeds most likely were brought in by wind or other dispersal mechanisms since the creation of the area in 1994. The remaining species in the seedbank (especially nasturtium, tall blue lettuce, flatsedge and St. John's wort) typically do not colonize disturbed areas, suggesting that the seeds may have been present in the seedbank prior to disturbance.

Table 4.1 Species composition of the JES upland seedbank. Soil for this experiment was collected June, 1995.

Latin name	Common name	Origin/ Habitat*
Chenopodium album	pigweed	A/D
Conyza canadensis	horseweed	N/D
Crepsis tectorum	hawkweed	A/D
Cyperus squarrosus	flatsedge	N/U
Hypericum majus	St. john's wort	N/U
Lactuca biennis	tall blue lettuce	N/U
Lepidium virginicum	poor man's pepper	N/D
Oxalis acetosella	wood sorrel	N/D/U
Plantago major	common plantain	?/D
Rorippa nasturtium-aquaticum	nasturtium	A/U
Rorippa palustris	yellow water cress	N/U
Silene latifolia	white campion	A/D

* Abbreviations: N= a plant NATIVE to Minnesota; A= a plant ALIEN to Minnesota; U= a plant typically found on UNDISTURBED habitats; D= a plant typically found on DISTURBED habitats.

Table 4.2 can perhaps give more insight as to the influence of the seed bank on the vegetation community at the JES upland site. The seed most abundant in the soil is *Rorippa nasturtium-aquaticum* (nasturtium). Nasturtium, though originally native to Europe, has become so naturalized in North America that it is found extensively in undisturbed wetland habitats. Its presence in the seed bank of the upland site indicates that some of the seedbank in the upland was previously from a

wetland area. Given the well drained nature of the upland soils, however, it is unlikely that nasturtium will recolonize this area.

Table 4.2. Seed density of species present in the JES upland seedbank. Soil for this experiment was collected June, 1995.

Latin name	Common name	Mean # seeds/m^2 ± SEM
Rorippa nasturtium-aquaticum	nasturtium	3.4 ± 0.85
Chenopodium album	pigweed	0.56 ± 0.12
Oxalis acetosella	wood sorrel	0.56 ± 0.12
Conyza canadensis	horseweed	0.38 ± 0.22
Rorippa palustris	yellow water cress	0.25 ± 0.15
Silene latifolia	white campion	0.19 ± 0.12
Lactuca biennis	tall blue lettuce	0.06 ± 0.07
Lepidium virginicum	poor man's pepper	0.06 ± 0.07
Cyperus squarrosus	flatsedge	0.06 ± 0.07
Plantago major	common plantain	0.06 ± 0.07
Crepsis tectorum	hawkweed	0.06 ± 0.07
Hypericum majus	St. john's wort	0.06 ± 0.07

The next two most prevalent seeds in the upland soil are *Chenopodium album* (lamb's quarters) and *Conyza canadensis* (horseweed). Both of these are common weeds throughout our area and most likely represent seed that has blown in since the creation of the site. Overall, the species found in the seedbank were quite diverse, and many of the less common species would be desirable recruits in a restoration effort. Consequently, the seedbank could represent a significant factor in the re-establishment of native plant communities. This would especially true when no seeding of native plants is done.

The results of the wetland seedbank study are presented in Table 4.3. The most prevalent species is *Epilobium ciliatum* (American willow herb). Like many of the species present in the upland seedbank, American willow herb is native to Minnesota but is often found in disturbed habitats. *Lemna minor* (duckweed) is present almost exclusively in the flats that are maintained under constantly saturated conditions, and in nature is found in both disturbed and undisturbed sites. Since little planting was done in the wetland at the JES site, the seed present in the

seed bank will give valuable insight to the types of plant communities that become established.

Table 4.3. Species compostion of the JES wetland seedbank. Soil for this experiment was collected August, 1995.

Latin Name	Common Name	Origin/ Habitat
Epilobium ciliatum	American willow herb	N/D
Epilobium parviflorum	Eurasian willow herb	A/D?
Lemna minor	duckweed	N/U and D
Bidens cernua	common beggar's tick	N/D
Plantago major	common plantain	?/D

Abbreviations: N= a plant NATIVE to Minnesota; A= a plant ALIEN to Minnesota; U= a plant typically found on UNDISTURBED habitats; D= a plant typically found on DISTURBED habitats.

4.3.2 Revegetation in the upland plots

Three months after seeding in the JES upland few planted species were observed. Only common ox-eye, black-eyed susan, and little bluestem were seen, all of which at low abundance. The majority of common species were naturally recruited, and considered to be weedy. Two species, horseweed and plantain, were found in the seedbank, whereas Kentucky bluegrass and crab grass seed must have established from windborne seed. Two cover crop species, oats and Regreen, still comprised a fair portion of cover as well.

Table 4.4 Species compition of the JES upland prairie area, fall 1995. Species are listed in order of decreasing abundance. Species in the first group were recorded at greater than 10% cover, species in the second group had 1 to 10 percent cover, and species in the third group had less than 1% cover.

Latin name	Common name	Planted	Seedbank	Cover crop
Poa pratensis	Kentucky bluegrass			
Avena sativa	oats			X
Conyza canadensis	horseweed		X	
Triticum aestivum X *Elymus trachycaulus* (sterile)	Re-Green			X
Digitaria ischaemum	crab grass			
Plantago major	common plantain		X	
Lolium perenne var. *aristatum*	annual rye			X
Setaria sp.	foxtail			
Silene pratensis	white campion		X	
Vicia sp.	vetch			
Melilotus officianalis	yellow sweet clover			
Trifolium sp.	red clover			
Brassicaceae sp. 1	---			
Ambrosia artemisiifolia	ragweed			
Fragraria sp.	strawberry			
Heliopsis helianthoides	common ox-eye	X		
Oxalis acetosella	wood sorrel		X	
Rudbeckia hirta	black-eyed susan	X		
Schizachyrium scoparium	little bluestem	X		
Trifolium sp.	clover			
Brassicaceae sp. #2	---			
Polygonum sp.	smartweed			
Brassicaceae sp. #3	---			
Medicago sativa	alfalfa			

By fall 1996, 15 months after planting, the planted species were much more common (Table 4.5). This is particularly true of the native grasses, which were among the most common species observed at the site. Some of the naturally recruited weeds disappeared entirely (for example, second year horseweed seedling growth is inhibited by decaying root material from the first year [31]). In contrast to the seeded grass species, many of the seeded forbs were still not apparent at this time. Even after 15 months, only 3 of the 21 planted forb species were noted. Ongoing studies at the site will indicate whether the other 18 species will eventually germinate, or if the seeding of these species was unsuccessful. These results suggest, however, that a greater percentage of forb seed may be needed in the mix to result in significant forb representation.

Table 4.5 Species composition of the JES upland prairie area, fall 1996. Species are listed in order of decreasing abundance. Species in the first group were recorded at greater than 10% cover, species in the second group had 1 to 10 percent cover, and species in the third group had less than 1% cover.

Latin Name	Common Name	Planted	Seedbank	Cover crop
---	warm season grasses*	X		
Lolium perenne var. *aristatum*	annual rye			X
Schizachyrium scoparium	little bluestem	X		
Andropogon gerardii	big bluestem	X		
Digitaria ischaemum	crab grass			
Poa pratensis	Kentucky bluegrass			
Melilotus sp.	clover			
Agrostis gigantea	red top			
Rudbeckia hirta	black-eyed susan	X		
Potentilla sp.	cinquefoil			
Vicia sp.	vetch			
Setaria sp.	foxtail			
Phleum pratense	timothy			
Phalaris arundinacea	reed canary grass			
Bouteloua curtipendula	side oats grama	X		
Medicago sativa	alfalfa			
Trifolium hybridum	alsike clover			
Silene pratensis	white campion		X	
Elymus canadensis	Canada wild rye	X		
Elymus trachycaulus	slender wheat grass	X		
Triticum aestivum X *Elymus trachycaulus* (sterile)	Re-green			X
Ambrosia artemisiifolia	ragweed			
Panicum sp.	panic grass			
Trifolium sp.	red clover			
Brassicaceae sp.	---			
Panicum miliaceum	broomcorn millet			
Graphalium obtusifolium	sweet everlasting			
Heliopsis helianthoides	common ox-eye	X		

* This category consisted of vegetative *Andropogon gerardii* (big bluestem), *Sorghastrum nutans* (Indian grass), and *Panicum virgatum* (switch grass), which could not be distinguished.

4.3.3 Revegetation at JES pond

As can be seen from the list of species at the JES wetland (Table 4.6), there is a great amount of plant diversity present considering the age of the site. Forty-nine plant species were identified along the northwest shore of JES pond, representing 20 different plant families. Most of the species observed were found in adjacent wetlands, and apparently recruited naturally from these areas. Some of these areas are connected hydrologically so that plant species whose seeds are dispersed via water (e.g. the sedges) could reach the JES site. Eight of the species planted along the

south and east sides of the pond were found on the unplanted northwest side of the pond, but 7/8 of these species were also found in adjacent wetlands, so the ultimate propagule source is impossible to pinpoint. The success of the plantings as propagule sources cannot be evaluated at this time. Similarly, only 2 of the observed species on the northwest side of JES were also found in the seedbank, and both of these species were also found in adjacent wetlands.

Table 4.6 Species composition of the JES wetland area, 1996. Vegetation surveys were conducted along the north and northwest sides of JES pond.

Latin name	Common name	Planted[a]	Seedbank[b]	Adjacent wetlands[c]
Agrostis stolonifera	red top			X
cf. Agrimonia sp.	agrimony			
Alisma plantago-aquatica	water plantain			X
Asclepias incarnata	swamp milkweed	X		X
Carex bebbi	sedge			
Carex comosa	sedge	X		
Carex hystericina	sedge			X
Carex lanuginosa	sedge			
Carex retrorsa	sedge			X
Carex scoparia	sedge			
Carex stipata	sedge			
Carex vulpinoidea	sedge			X
Cicuta bulbifera	bulbiferous water hemlock			X
Cyperus bipartitus	flatsedge			
Eleocharis accicularis	spike rush			
Eleocharis cf. ovata	blunt spike-rush			
Elymus canadensis	Canada wild rye			
Epilobium parviflorum	willow herb		X	X
Eupatorium maculatum	spotted joe-pye weed	X		X
Eupatorium perfoliatum	boneset	X		X
Gerardia sp.	agalinis			X
Glyceria grandis	American mannagrass	X		X
Juncus brevicaudatus	rush			
Juncus effusus	soft rush			X
Juncus nodosus	rush			
Juncus tenuis	path rush		X	X
Leersia orzoides	rice cutgrass			X
Lobelia inflata	Indian tobacco			
Lolium perenne var. *aristatum*	Italian ryegrass			
Lycopus americanus	bugleweed			X

Table 4.6 Composition of the JES wetland area, 1996, continued.

Latin name	Common name	Planted[a]	Seedbank[b]	Adjacent wetlands[c]
Melilotus alba	white sweet clover			X
Melilotus officinale	yellow sweet clover			X
Mimulus ringens	Allegheny monkeyflower	X		X
Pentharum sedoides	ditch stone-crop			X
Phalaris arundinacea	reed canary grass			X
Phleum pratense	timothy			X
Polygonaceae sp.	--			X
Potentilla sp.	strawberry weed			
Ranunculus cf. flabellaris	yellow water crowfoot			
cf. Rorippa sp.	nasturtium			
Rudbeckia hirta	black-eyed susan			X
Rumex crispus	curly dock			
Salix sp.	willow			
Scirpus atrovirens	bulrush	X		X
Scirpus cyperinus	bulrush			X
Scirpus validus	bulrush	X		X
Setaria glauca	foxtail			
Typha cf. latifolia	cattail			X
Verbena hastata	common vervain			X

[a] An X in this column indicates that the species was planted at the south side of JES pond in 1995.
[b] An X in this column indicates that the species was found in the wetland seedbank study.
[c] An X in this column indicates that the species was found in vegetation surveys of at least one of the adjacent wetlands.

In this particular instance, the vegetation at the surrounding sites is a better indication of the potential plant community at JES than the seedbank study. In future restoration efforts, this factor should be taken into consideration. A restoration site with relatively undisturbed areas nearby will likely be covered with native, desirable vegetation, even if relatively little seeding is done. However, an isolated restoration area would not have this seed source, and would likely have a very different outcome. Successful restoration of isolated areas would be far more dependent on plantings, seeding, and the seedbank for sources of propagules.

Chapter 5. Mycorrhizal Parameters of Three Wetland Plant Species at Undisturbed Wetland Sites.

5.1 Overview

AM fungi are known to benefit plants by increasing the uptake of nutrients, especially phosphorus [32], increasing drought tolerance [33] and potentially protecting roots from plant pathogens [34]. Comparatively few studies, however, have examined the presence or significance of AM fungi in wetland plants and those studies have often been contradictory. Kahn [35], for example, found no root colonization in 16 hydrophytes and suggested that the wet conditions resulted in a lack of AM fungal colonization. Likewise, Gerdemann [36] found AM fungi in rice growing in dry soils but rice growing in flooded soils lacked AM fungi. More recently, however, AM fungi have been discovered in the roots of cattails (*Typha* spp.) [11], in rice in flooded soils [37], and in plants in prairie pothole wetlands [38, 39, 40]. The presence of a functional mycorrhizal relationship frequently judged by the appearance of arbuscules in the roots of the host plant. In wetland plants, arbuscules have been absent [11, 40, 41] or present in very low amounts [42].

It has been suggested that AM fungi act as benign parasites in soil that is saturated but may become mutualistic in unsaturated soil [38, 41, 43]. Mejstrik [44, 45] found that colonization in bog plants was dependent on depth of the water table. On the other hand, Wetzel and van der Valk [40] reported no relation of percent colonization to soil moisture. Intensity of infection may also be related to the nutrient status of the wetland or aquatic system. Tanner and Clayton [42] and Chaubal et al. [46] both found higher colonization levels in plants growing in low nutrient sediments compared to high nutrient sediments. Clayton and Bagyaraj [47], on the other hand, could not relate AM fungal infection with the trophic status of the system.

Given this conflicting evidence, the factors controlling AM fungal colonization in wetland plants are poorly understood. Research in this area is complicated by the fact that wetlands are dynamic systems. Complex changes in nutrient cycling, nutrient availability and presence of oxygen often accompany

seasonal water table changes. Furthermore, different plant species exhibit differing degrees of mycotrophy, making generalizations along a moisture gradient difficult.

In order to reach a better understanding of the presence and function of mycorrhizae in a wetland restoration, undisturbed wetland sites at Cedar Creek Natural History Area (CCNHA) were monitored for mycorrhizal parameters and soil conditions. Three wetland plant species were chosen because of their widespread distribution in the Anoka sand plain and their possible ecological importance in wetland restorations at roadside reclamation sites: blue joint grass (*Calamagrostis canadensis*), sedge (*Carex lasiocarpa*), and the invasive weed reed canary grass (*Phalaris arundinacea*). The information gained from these species will then be analyzed to determine colonization rates under different wetland environmental conditions.

5.2 Materials and methods

Three undisturbed wetland sites were selected at CCNHA, which is located in Isanti County at 45° 42′ N latitude and 93° 19′ W longitude. The soils were mapped as poorly drained Isanti loamy fine sands. Cedar Creek was chosen because, like the Cambridge re-creation complex, it is located on the Anoka sand plain, and is therefore geologically and edaphically similar. Each wetland site has areas dominated by either *Phalaris arundinacea* (reed canary grass), *Carex lasiocarpa* (sedge), or *Calamagrostis canadensis* (blue joint grass). Each site also has zones where the dominant species present grows in upland, transitional and wetland areas (Figure 5.1). At each site, three replicate plots measuring $2m^2$ were established in each zone, yielding a total of nine plots per plant species. Nine soil cores were taken from each plot on 10-25-95 using a 5 cm diameter corer to a depth of 6 cm. This soil was used for nutrient analyses, root colonization and mycorrhizal spore analysis. For *Phalaris* only, individual plants were also collected from each plot adjacent to each soil core by clipping at the base of the plant.

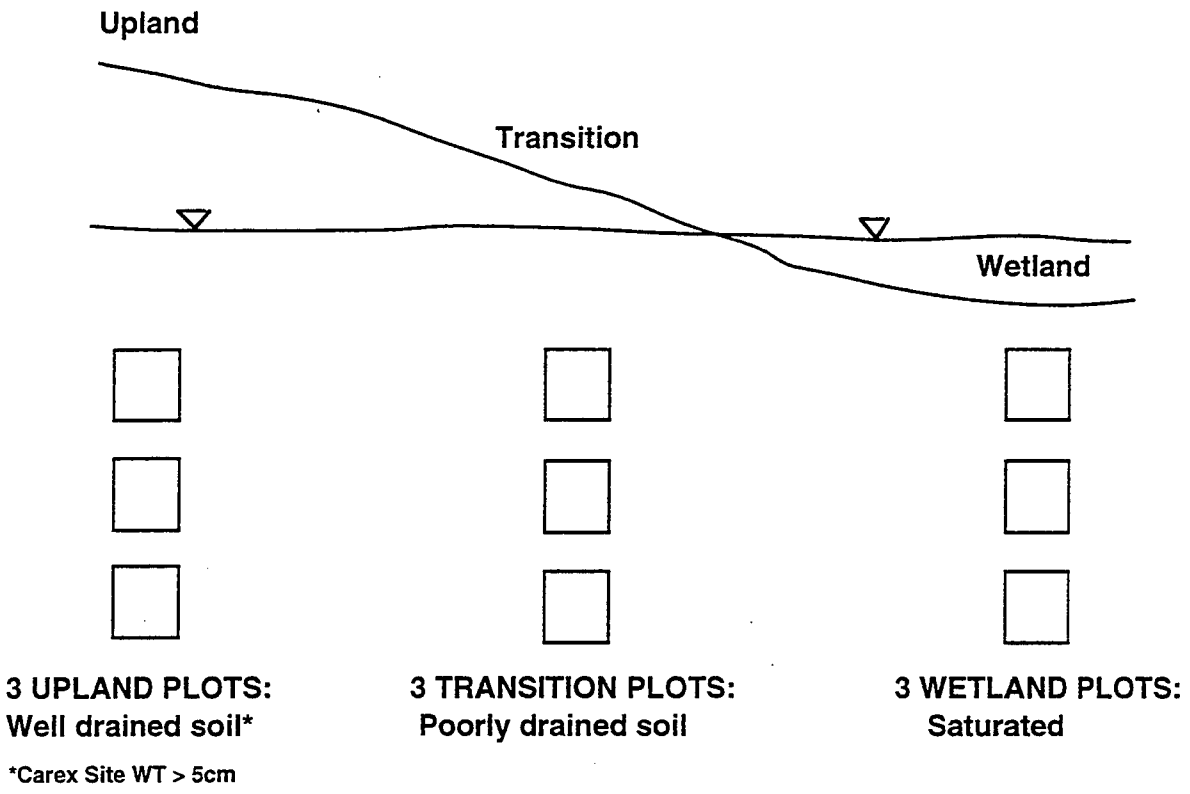

Figure 5.1. A schematic diagram of plots at each site along a moisture gradient. The horizontal line represents the water table. *Calamagrostis, Phalaris,* and *Carex* sites were all located at Cedar Creek Natural History Area, Isanti County, Minnesota.

5.2.1 Mycorrhizal analysis

Roots were immediately isolated from soil cores over a 250 micrometer sieve, washed free of debris with tap water and preserved in 50% ethanol. Clearing and staining procedures followed methods modified from Phillips and Hayman [24], Kormanik *et al.* [25], and Koske and Gemma [26]. Roots obtained from the upland zones were cleared overnight in 10% KOH, acidified in 1% HCl, stained overnight with 0.05% trypan blue and destained with acidic glycerol. It was found that wetland roots were much more fragile than upland and transition roots. The clearing and staining times were therefore reduced to 7 hours each. Clearing and staining for longer times often resulted in roots that were over digested or over stained. A randomly selected subsample of the stained roots from each plot were mounted on microscope slides. Percentage AM fungal colonization was determined using the

magnified intersection method [27]. Roots were examined at 160X and 400X. Four replicates of 100 intersections each were counted for each plot. Each intersect was evaluated for the presence of mycorrhizal structures: arbuscules, vesicles, or AM hyphae. AM hyphae were distinguished from hyphae of unknown origin by the presence of mycorrhizal structures attached to them. If the eyepiece crosshair intersected a hypha that was attached to a vesicle or arbuscule within the field of view, this hyphae was counted as an AM hypha.

Spore density was also measured for a subset of the plots. Spores were isolated using a sucrose density centrifugation protocol modified from Tommerup and Kidby[20] and Daniels and Skipper [21].

5.2.2 Soil and plant tissue analyses

The soil cores taken from each plot at each sampling period were analyzed for soil moisture, pH, phosphorus, ammonium, and nitrate. Soil pH was measured using 0.01M $CaCl_2$ solution with a ratio of 1 g soil : 2 ml $CaCl_2$ for mineral soils and 1 g soil : 4 ml $CaCl_2$ for organic soils. Data on percent moisture of soil was collected at time of sampling. Soil cores for phosphorus and pH analysis were dried whereas cores for ammonium and nitrate analysis were frozen immediately after collection until time of testing. The analysis of soil phosphorus was done at the University of Minnesota Research and Analytical Laboratories using Bray-P test. Nitrate and ammonium were extracted using 2M KCl and analyzed with an autoanalyzer following methods outlined in Maynard and Kalra [48]. Plant tissue was ground and sent to the University of Minnesota Research and Analytical Laboratories for inductively coupled plasma atomic emission spectroscopy (ICP) testing of plant tissue content.

5.3 Results and discussion

5.3.1 Mycorrhizal analysis

Figure 5.2 shows the mycorrhizal colonization of roots at each of the sites. For all species, roots from the upland zone had the greatest percent colonization, with lower levels of colonization in roots from the transition and wetland zones.

These differences were significant for hyphal colonization of the *Phalaris* and *Carex* roots.

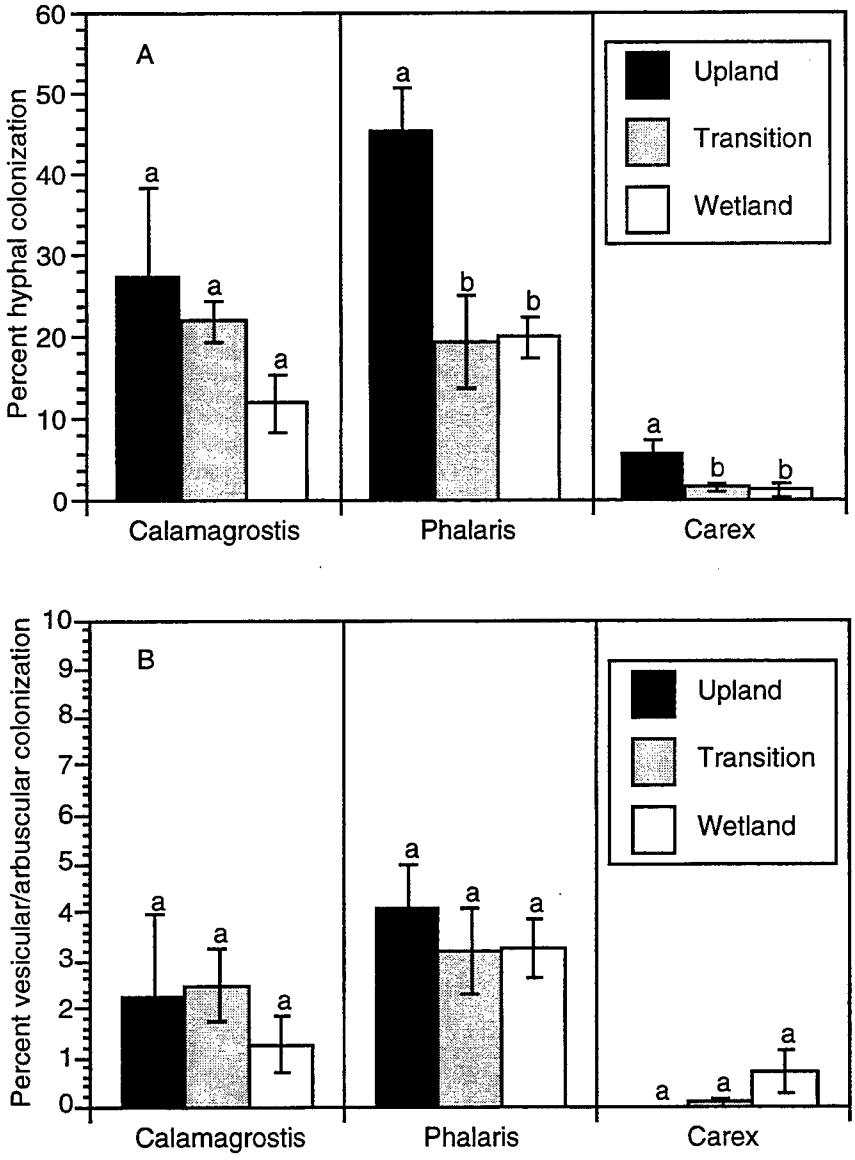

Figure 5.2 Mycorrhizal colonization of 3 wetland species growing at upland, transition or wetland zones. Figure A depicts hyphal colonization, and figure B depicts vesicular /arbuscular colonization.

These results support the hypothesis that more water will result in lower levels of mycorrhizae. Many other studies have shown a similar inverse relationship between water table and AM colonization (38, 44, 49). This conclusion is further supported by the fact that *Carex*, the species that grows in the most

saturated conditions (Table 5.1), had the lowest mycorrhizal levels. Even the upland zone of the *Carex* site was characterized by greater than 75% soil moisture.

Table 5.1 Soil characteristics of upland, transition, and wetland soil at wetland sites dominated by three plant species.

plant species	zone	pH	Bray-P (ppm)	NH_4-N (ppm)	NO_3-N (ppm)	% moisture
Calamagrostis	Upland	4.2	7	7.7	0.7	22.9
	Transition	4.0	23	20.7	0.6	70.8
	Wetland	3.9	30	28.9	0.5	79.1
Phalaris	Upland	5.0	72	41.4	1.0	36.7
	Transition	4.7	61	21.5	0.5	72.7
	Wetland	4.5	72	27.8	0.6	74.0
Carex	Upland	4.2	42	10.4	0.6	75.9
	Transition	4.4	12	9.0	0.5	82.4
	Wetland	4.4	20	21.0	0.5	84.8

Consistent with other published results [11, 40, 41, 42], overall vesicular/arbuscular colonization of these roots was low, particularly in the wetland zones.

In contrast to root colonization, spore density was greatest in the transition zone, lowest in the wetland zone, and intermediate in the upland zone for all plant species (Figure 5.3). As with root colonization, the lowest spore numbers were found at the *Carex* site. These results suggest that wetter (but not saturated) conditions may encourage spore formation in some AM fungal species. This in turn may affect plant health in seasonally wet areas like those present on the margin of the JES restoration pond. Further research is needed to determine the precise effect of moisture level on AM fungal spore formation.

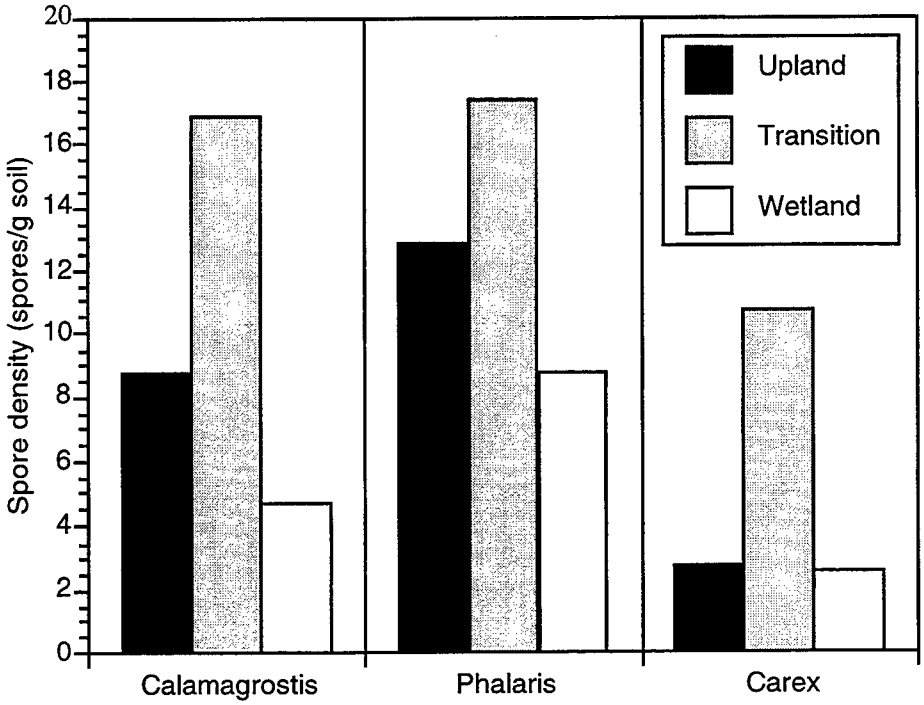

Figure 5.3 Mycorrhizal spore densities of soil collected from sites dominated by 3 wetland species. For each site, soil was collected from upland, transition, and wetland zones.

5.3.2 Soil and plant tissue analyses

Not surprisingly, percent moisture increased from the upland to transition to wetland zones for each species (Table 5.1). Generally, there was little difference between soil moisture in the transition and wetland zones, but percent soil moisture in the upland zone was much lower. At the *Carex* site, however, even the upland site had high soil moisture, only a little drier than the other two zones.

Soil pH was negatively correlated with soil saturation at sites, such that increased soil saturation was generally associated with lower soil pH. It is possible, therefore, that lower pH may also have contributed to the lower AM fungal colonization in the wetland zones. AM fungi have been shown to behave differently in soils with different pH [14, 15].

Soil phosphorus tended to be highest in the upland for all species, although the *Phalaris* sites had equal amounts of phosphorus available in the wetland zone, and only slight differences among zones (Table 5.1). A major role of AM fungi in

terrestrial systems is in increasing the uptake of phosphorus [50]. In this case, however, the zones with most phosphorus also had greatest mycorrhizal colonization, suggesting that water saturation, or some correlated characteristic, is a more driving factor than soil phosphorus in determining mycorrhizal colonization levels. Phosphate availability in wetlands is directly dependent on the supply of free oxygen and redox potential [51, 52]. Soils that are submerged generally have higher levels of available phosphorus [53]. This effect may be counteracted by the exudation of oxygen into the rhizosphere of the wetland plants which creates an oxidized zone around the plant roots. In this type of environment, ferrous iron is oxidized to ferric iron which readily complexes with phosphate and may make it unavailable to the plant [54]. These complex dynamics are reflected in literature, which fails to show consensus. Soil phosphorus has been found to be both correlated [40] and not correlated [38] with AM fungal colonization along a moisture gradient.

Plant tissue analysis of *Phalaris* shows that plants in the wetland zone had significantly greater phosphorus than plants in the other 2 zones, even though soil phosphorus was the same between upland and wetland, and plants in the upland zone had greater levels of AM fungal colonization (Table 5.1). Although the phosphorus tissue content of *Phalaris* is well below the adequate range reported for other grass species [55] it is within the range reported for *P. arundinacea* [56] indicating that these plants were probably not limited by phosphorus.

Nitrogen was primarily represented as ammonium at all sites in all zones, with little nitrate present (Table 5.1). This result is typical of saturated soils [57]. The availability of nitrogen among zones showed strikingly different patterns among the sites. At the *Calamagrostis* site, nitrogen was lowest in the upland and highest in the wetland. The *Carex* site also had the highest nitrogen under wetland conditions. The *Phalaris* site, on the other hand, had the highest nitrogen in the upland. Nitrogen is often the most limiting nutrient in flooded soils [53], and AM fungi have been shown to produce enzymes which allow the assimilation of ammonia [58, 59]. Thus, it is possible that plants in flooded soils may benefit from increased nitrogen nutrition when colonized by AM fungi. However, we found low

colonization under saturated conditions, making it unlikely that the plants are receiving such a benefit.

Chapter 6. Mycorrhizal diversity of undisturbed Minnesota prairies

Relative to other taxa, reported global species diversity for AM fungi is relatively low [60]. There are ~250,000 described species of plants, ~5000 species of ectomycorhizae, but less than 200 species of AM [61, 62]. It has been argued that this apparent lack of diversity indicates lack of host specificity in AM fungi [60, 63]. However, this should not be taken as evidence that AM fungal diversity is unimportant. Several studies have found differences in the benefits conferred to plants by different AM species [16-19]. Moreover, using intensive sampling and culturing techniques, Bever *et al.* [64] observed 23 distinct AM fungal species at a single site, seven of which were previously undescribed. This suggests that AM fungal diversity may be underrepresented in the literature.

The purpose of this chapter is to begin characterizing the mycorrhizal communities of native remnant prairies, for comparison to the community at the restored prairie at JES. Our eventual goal is to determine whether a comparable mycorrhizal community is developing in the restored site. This chapter describes the first step in the process; analysis of AM fungal community composition at JES and three remnant prairie areas using spore morphology data (the Calamagrostis site at Cedar Creek Natural history area, Feder prairie, and Crosstown prairie). While somewhat crude, spore morphology data can give a rough overview of whether the communites are similar or not. True characterization of the communities will require long-term intensive work, involving mycorrhizal culturing and spore identification [65]. Some preliminary work on these tasks has been undertaken at the Crosstown site.

6.2 Materials and Methods

6.2.1 Spore morphotype analysis of field communities

At the Calamagrostis upland site three soil cores were taken from each of three replicate $2m^2$ plots on 10-25-95 using a 5 cm diameter corer to a depth of 6 cm. At the 12 JES upland plots (see Chapter 2 for details), 25 soil cores were taken from each plot on 9-12-95 using a 3/4" soil probe to a depth of 6 cm. All 25 cores were thoroughly homogenized, soil was dried, and spores isolated from a sub-sample of

the soil using a sucrose density centrifugation protocol modified from Tommerup and Kidby[20] and Daniels and Skipper [21]. Spores were counted and classified by morphotype under a dissecting microscope at 30-50X magnification.

Data from Crosstown and Feder remnant prairies is reproduced from Charvat *et al.* [4]. Soil from Crosstown prairie was collected on 10-8-94, and soil from Feder prairie was collected on 11-12-94. Spore isolation and counting procedures were the same as for the Calmagrositis and JES upland sites.

6.2.2 Spore identification from Crosstown prairie

Characterization of the mycorrhizal community at Crosstown prairie has been an ongoing process. While spores can occasionally be identified from field samples, in most cases laboratory propagation of field material is necessary to obtain sufficient quantities of spores for taxonomic identification. General "trap cultures" of Crosstown soil have been performed under controlled conditions in the laboratory using two native grasses, big bluestem (*Andropogon gerardii*) and side-oats grama (*Bouteloua curtipendula*), as hosts. Additionally, pot cultures of individual spore morphotypes were set up using big bluestem as a host. In both types of cultures, plants were allowed to grow in a mixed soil/sand medium in the growth chamber, watered 2-3 times per week. After 4 months, the plants were allowed to senesce, and the soil was placed in cold storage until spore isolation and identification could be undertaken.

Mycorrhizal spores were isolated from the soil using sucrose density centrifugation [20,21]. Individual spores were tranferred to clean petri dishes with a pipette. Spore charateristics examined included size (measured with an ocular micrometer), color (based on INVAM color designations), subcellular spore characters, and reaction of spore wall to Melzer's reagent [66]. Taxonomic identification was based upon species descriptions in Schenk and Perez [67] with modifications provided by INVAM [66]. Type specimens for each spore type were preserved in vials in 0.05% sodium azide, and on slides.

6.3 Results and Discussion

Table 6.1 presents spore morphotype distribution at three remnant prairies and the JES study site. The overall spore numbers found at the three native remnant prairies were comparable, with spore densities of 17-20 spores per gram of soil. Spore density was much lower at JES, averaging only 1.5 spores per gram of soil. It must be borne in mind that this value reflects spore densities at only 6 months after seeding of the restoration, when the vegetation was still largely composed of non-mycorrhizal ruderal species (see Ch 5). Consequently, low mycorrhizal presence and low spore density would be unsurprising at this stage of the restoration. On the other hand, mycorrhizal spore production and mycorrhizal colonization are not always well correlated [68], and lack of spores should not be considered indicative of lack of mycorrhizae. Indeed, the relatively high root colonization values from fall 1996 at this site indicate a strong mycorrhizal presence, despite the low spore densities.

Table 6.1 Spore morphotype distribution at undisturbed prairies, in comparison to the JES upland restoration site.

	Site			
spore morphotype	Cedar Creek-Calamagrostis upland site	Crosstown remnant prairie [a]	Feder remnant prairie [a]	JES restoration site[b]
large brown	0.2 ± 0.04	1.6 ± 0.6	1.1 ± 0.2	0.1 ± 0.0
small brown	15.3 ± 8.1	3.8 ± 2.8	13.4 ± 2.5	0.2 ± 0.0
large reddish	0.1 ± 0.03	0.6 ± 0.2	0.1 ± 0.1	0 ± 0
small reddish	0.8 ± 0.2	1.1 ± 0.4	0.1 ± 0.1	0 ± 0
large yellow	0.3 ± 0.2	0.3 ± 0.1	0 ± 0	0.4 ± 0.0
small yellow	0.2 ± 0.1	11.3 ± 6.4	0.1 ± 0.1	0.2 ± 0.0
large hyaline	0.0 ± 0.0	0.1 ± 0.0	0.5 ± 0.3	0.5 ± 0.2
small hyaline	1.2 ± 0.1	0.7 ± 0.7	2.6 ± 0.6	0.2 ± 0.0
black	0 ± 0	0.3 ± 0.2	0 ± 0	0 ± 0
green	0 ± 0	0.2 ± 0.1	0 ± 0	0 ± 0
Total	18.03 ± 8.22	20.2 ± 5.7	17.8 ± 2.8	1.5 ± 0.1

[a] data from Charvat *et al.* [4].

[b] averaged over all treatments (inoculated, uninoculated soil, and uninoculated; there were no significant differences among treatments).

Table 6.2 lists some of the mycorrhizal species identified from Crosstown remnant prairie. Under previous funding from LCMR, eight other mycorrhizal species have been identified: *Glomus occultum, Glomus intraradices, Glomus mosseae, Glomus etunicatum, Glomus constrictum, Gigaspora gigantea, Scutellospora pellucida,* and *Entrophospora infrequens.* Additionally, at least six mycorrhizal species remain unidentified, and possibly represent undescribed species.

Table 6.2 Mycorrhizal species identified from Crosstown prairie

Mycorrhizal species
Acaulospora spinosa
Glomus leptoticum
Gigaspora margarita
Scutellospora fulgida
Scutellospora coralloidea
Scutellospora calospora

These results indicate that, similar to the findings of Bever *et al.* [64], estimates of mycorrhizal species diversity are largely a function of effort invested. Successive pot cultures and use of a variety of hosts will result in a more complete picture of the mycorrhizal community. Further effort in characterizing mycorrhizal diversity at Crosstown is being funded under an ongoing Mn/DOT grant, and will be included in a future report.

Chapter 7 Production of mycorrhizal inoculum for incorporation into restoration sites

7.1 Overview

The primary objective of this study was to investigate production of spores for use at restoration sites. There were also a number of secondary goals to this experiment. The first goal was to determine the amount and quality of inoculum that could be produced by the "Beltsville system." The Beltsville system is a hydroponic method of culturing AM fungi and their plant hosts on a silica sand substrate [69]. In their original study, Millner and Kitt [69] found that AM fungal spore production was very high using this method and a corn host.

A second goal was to assess the use of a native prairie species as a host in mycorrhizal inoculum production. Big bluestem (*Andropogon gerardii* Vitman) was selected both because it is an obligate mycorrhizal species and for its overall hardiness [70]. A native species was used in hopes that it would best foster the reproduction of native prairie mycorrhizal spores. Big bluestem had been used as a mycorrhizal host in previous laboratory experiments [4], but never under a Beltsville culturing regime.

The final goal of this study was to test the efficacy of a liquid spore inoculum, rather than a more traditional soil inoculum. A liquid spore suspension would be much easier to apply in large scale restoration efforts, and storage of a concentrated liquid inoculum would be simpler than storage of bulky soil inoculum. To test the relative effectiveness of these two inoculation methods, three treatments were set up: pots that received soil inoculum, pots which received liquid spore suspension inoculum, and pots that received both types of inocula.

7.2 Materials and methods

7.2.1 Inoculum source

Soil and spore inocula for this experiment originated from the Crosstown remnant prairie site located within the city of Minneapolis. Crosstown prairie soil has previously been used to produce general AM fungal inoculum [4]. The soil

inoculation treatment received 51.7 g of this inoculum, corresponding to ~500 spores, plus infective root and hyphal segments. The liquid inoculation treatment consisted of a liquid suspension of spores produced from multiple spore isolations of the Crosstown general inoculum. To obtain the desired number of spores, 22 isolations were performed using a sucrose density centrifugation protocol, modified from Tommerup and Kidby [20] and Daniels and Singer[21]. Isolated spores were suspended in water and stored at 4° C until use. Immediately prior to use, the suspension was diluted to approximately 500 spores per 5 ml of water. The combination inoculum treatment received both 51.7 g of soil and 5 ml of spore suspension. Inoculum soil and spore suspension controls were prepared by sterilizing them at 121°C for 20 minutes.

7.2.2 Experimental set-up and maintenance

Black plastic pots 14.5 cm in diameter were filled 11 cm deep with moist silica-sand microspheres. Pots receiving liquid inoculum had 2 ml of spore suspension added directly to the sand surface. Soil treatment pots had 51.7 grams of soil added evenly to the pot. An average of 348 imbibed and sterilized big bluestem seeds were added (by weight) to all pots. The remaining 3 ml of spore suspension were then added to pots receiving the liquid suspension treatment. A 1.5 cm layer of sand was placed over the big bluestem seeds. Fifteen replicates were set up for each treatment. An additional five control pots were set up for each treatment using sterilized inoculum rather than viable inoculum. For all pots, drip irrigation watering rings were placed directly on the sand, and pots were temporarily covered with plastic to prevent drying.

Pots were moved to the University of Minnesota, College of Biological Sciences Greenhouse. Plants were irrigated 5 times daily with approximately 65 ml of solution, using a protocol modified from Millner and Kitt [69]. Prior to emergence, plants were watered with tap water. Following emergence, a modified half strength Hoagland's nutrient solution was used (Table 7.1) [71]. All pots were supplied from the same reservoir of nutrient solution. After the first two weeks of growth, phosphorus concentration was limited to 10 μM, to encourage fungal

colonization. Fresh nutrient solution was added to a storage tank at approximately 3 day intervals. After 14 weeks, an additional 30 ml of solution was supplied to each pot during each watering period. Plants were maintained at ambient greenhouse temperatures from August 5th, 1996 until Jan 16th, 1997 (16 wks.). Average daily pot soil temperature fluctuated during the growing season from 60° to 90° F, with a top low and high of 55° and 105° F. In addition to naturally occurring light, artificial lighting was continuously supplied.

Table 7.1 Modified half-strength Hoagland's solution

chemical	concentration
$Ca(NO_3)_2*4H_2O$	2.5 mM
KNO_3	2.5 mM
$MgSO_4*7H_2O$	1.0 mM
NaFe EDTA (H_20)	0.05 mM
$CuSO_4*5H_2O$	0.5 µM
$CoCl_2*6H_2O$	0.2 µM
$NiSO_4*6H_2O$	0.2 µM
H_3BO_3	10.0 µM
$MnCl_2*4H_2O$	2.0 µM
$ZnSO_4*7H_2O$	1.0 µM
$NaMoO_4*2H_2O$	0.2 µM
HCL 3N	As needed
KOH	As needed
KH_2PO_4	10.0 µM
MES buffer	0.5 µM

7.2.3 Harvest and analysis

At the end of the growing period, stems were removed from all plants and separately placed in paper bags and dried at 65° C for biomass analysis. During harvest, plants were scored for symptoms of nutrient or other deficiency. All control plants (15 replicates) and 3 replicates from each treatment were used to calculate root biomass and fungal colonization. Pots with plants selected for root analysis were carefully emptied of the root mass and sand. Sand was cleaned from the roots, and the root mass was divided in half. One half was dried in a 65°C drying oven, and the other half was stored in 50% ethanol for later colonization analysis.

All pots not selected for immediate work were returned to the greenhouse growing room and allowed to dry, for future use as an inoculum source.

To evaluate inoculum production, two methods were used. First, spores were isolated from 15 grams of soil from each pot, and quantified under a dissecting microscope. Second, AM fungal colonization of the roots was determined. Roots stored in 50% ethanol were cleared in for 7.5 minutes in 10% KOH at 90° C , then acidified with 1% HCl for 1 hr at 90°C and stained with trypan blue. Percent colonization of the roots was determined using the magnified intersect method [27], previously described in section 2.5.1.

7.3 Results and discussion

Throughout the experiment, plant growth was rapid. Big bluestem thrived under the Beltsville watering system, achieving 2 to 3 times greater size than typically found under standard soil cultivation. This growth may reflect higher levels of nutrient availability under the hydroponic system. However, differences were noted among treatments in terms of the overall health of the plants. Control plants and plants which received only the liquid inoculum were anthocyatic, a symptom of characteristic of phosphorus deficiency [72]. Plants which received soil inoculum were much greener and fuller, and lacked visual signs of phosphorus deficiency. Since mycorrhizal symbiosis increases phosphorus availability to the plant, this result implies that plants which received only the liquid inoculum may not have been colonized by AM fungi, and that the liquid spore suspension may not have been an adequate inoculum.

Replicates which received only the liquid inoculum also produced significantly fewer spores than either treatment which received soil inoculum (Fig. 7.1). Spore density for the liquid inoculum treatment was less than half that in either of the other two treatments, and not significantly different than zero. At least three mycorrhizal fungal species have been identified from the inoculum produced in this experiment: *Glomus mosseae, G. etunicatum,* and *G. occultum.*

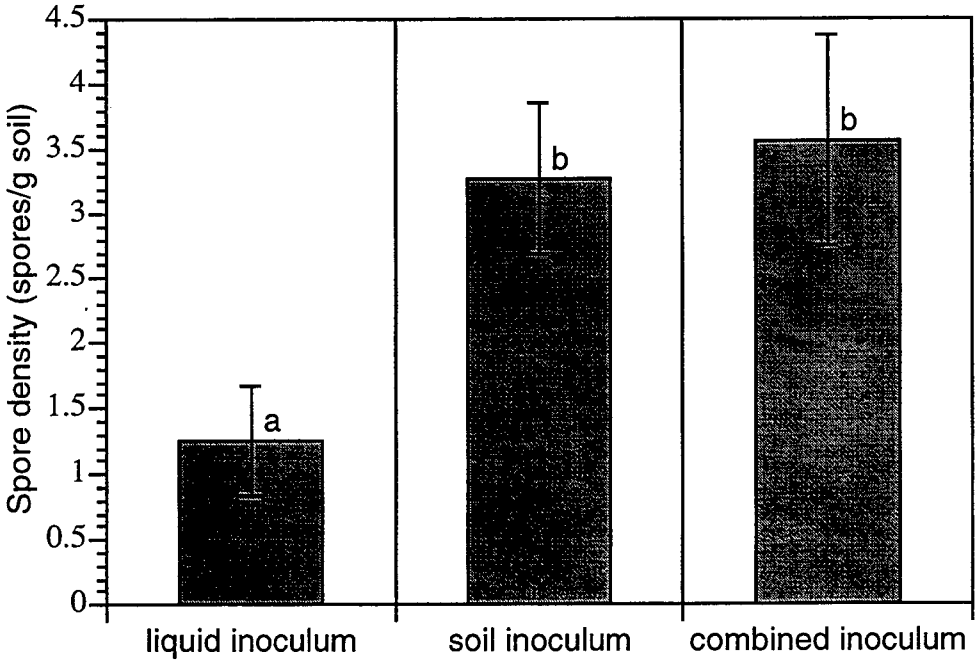

Figure 7.1 Spore density produced under cultivation using three different inoculation treatments: liquid spore suspension, soil inoculum, or both. Columns with the same letter were not significantly different at $\alpha = 0.05$.

Overall, spore production was low in all treatments. However, qualitative observations of root colonization showed that roots which received soil inoculum were heavily colonized by AM fungi. Roots from the soil inoculum treatment and the soil + liquid inoculum treatment appeared to have large quantities of AM fungal hyphae, vesicles and arbuscules, whereas AM fungi were virtually absent from roots which received only the liquid inoculum. Quantification of fungal colonization is being funded under an ongoing Mn/DOT grant, and will be included in a future report. The inoculum produced in this experiment was applied as whole inoculum to a restoration site at the Mn/DOT Shakopee research facility, which will also be discussed in a final report to Mn/DOT, due in February, 2000.

References

1. Smith, S.E., and D.J. Read. 1997. Mycorrhizal Symbiosis. Academic Press, San Diego, CA. 605 pp.
2. Miller, R.M., and J.D. Jastrow. 1992. Extraradical hyphal development of vesicular-arbuscular mycorrhizal fungi in a chronosequence of prairie restorations. Pp. 171-176 *in* D.J. Read, D.H. Lewis, A.H. Fitter, and I.J. Alexander (eds.) Mycorrhizae in Ecosystems. C.A.B. International, Wallingford, UK.
3. Allen, E.B., and M.F. Allen. 1990. The mediation of competition by mycorrhizae in successional and patchy environments. Pp. 367-389 in D. Tilman and J.P. Grime (eds.) Perspectives on Plant Competition. Academic Press, San Diego, CA.
4. Charvat, I.D., T. Pawlowska, M. Smith, D. L. Stenlund, and K. Nichols. 1995. Re-introduction of soil mycorrhizae into roadside prairie plantings. Minnesota Department of Transportation report #96-16. Minnesota Department of Transportation, St. Paul, MN.
5. Schwab, S.M., and P.A. Loomis. 1987. VAM effects on growth of grasses in monocultures and mixtures. Proceedings of the North American Conference on Mycorrhizae, 7th. p. 264.
6. Hartnett, D.C., B.A.D. Hetrick, G.W.T. Wilson and D.J. Gibson. 1993. Mycorrhizal influence on intra- and interspecific neighbour interactions among co-occurring prairie grasses. Journal of Ecology 81: 787-795.
7. Francis, R., and D.J. Read. 1994. The contributions of mycorrhizal fungi to the determination of plant community structure. Plant and Soil 159: 11-25.
8. Sengupta, A., and S. Chaudhuri. 1990. Vesicular arbuscular mycorrhiza (VAM) in pioneer salt marsh plants of the Ganges river delta in West Bengal (India). Plant and Soil 122: 111-113.
9. Søndergåard, M., and S. Laegåard. 1977. Vesicular-arbuscular mycorrhiza in some aquatic vascular plants. Nature 268: 232-233.

10. Pond, E.C., J.A. Menge, and W.M. Jarrell. 1984. Improved growth of tomato in salinized soil by vesicular arbuscular mycorrhizal fungi collected from saline soils. Mycologia 76: 74-84.
11. Stenlund, D.S., and I.D. Charvat. 1994. Vesicular arbuscular mycorrhizae in floating wetland mat communities dominated by *Typha*. Mycorrhiza 4: 131-137.
12. Read, D.J., 1991. Mycorrhizas in Ecosystems. Experientia 47: 376-391.
13. Grime, J. P., J. M. L. Mackey, S. H. Hillier, and D. J. Read. 1987. Floristic diversity in a model system using experimental microcosms. Nature 328: 420-422.
14. Green, N.E., S.O. Graham, and N.C. Schenck. 1976. The influence of pH on the germination of vesicular-arbuscular mycorrhizal spores. Mycologia 68: 929-934.
15. Lambert, D.H., and H. Cole, Jr. 1980. Effects of mycorrhizae in establishment and performance of forage species in mine spoil. Agronomy Journal 72: 257-260.
16. Johnson, N.C., F.L. Pfleger, R.K. Crookston, S.R. Simmons and P.J. Copeland. 1991. Vesicular-arbuscular mycorrhizas respond to corn and soybean cropping history. New Phytologist 117: 657-663.
17. Johnson, N.C., P.J. Copeland, R.K. Crookston, and F.L. Pfleger. 1992. Mycorrhizae: possible explanation for yield decline with continuous corn and soybeans. Agronomy Journal 84: 387-390
18. Francis, R., and D.J. Read. 1995. Mutualism and antagonism in the mycorrhizal symbiosis, with special reference to impacts on plant community structure. Canadian Journal of Botany 73(Suppl 1): S1301-S1309.
19. Schenck, N.C., and R.A. Kinloch. 1980. Incidence of mycorrhizal fungi on six field crops in monoculture on a newly cleared woodland site. Mycologia 74: 445-446.

20. Tommerup, I.D., and D.K. Kidby. 1979. Appl. Environ. Microbiol. 37: 831.
21. Daniels, B. A., and H. D. Skipper. 1982. Methods for the recovery and quantitative estimation of propagules from soil. Pp. 29-35 *in* N. C. Schenck (ed.) Methods and Principles of Mycorrhizal Research. The American Phytopathological Society, St. Paul, MN.
22. Giovannetti, M., and B. Mosse. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist 84: 489-500.
23. Hanssen, J.F., T.F. Thingstad, and J. Goksoyr. 1974. Evaluation of hyphal lengths and biomass in soil by a membrane filter technique. Oikos 25: 102-107.
24. Phillips, J.M., and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55: 158-161.
25. Kormanik, P.P., W.C. Bryan, R.C. Shultz. 1980. Procedures and equipment for staining large numbers of plant roots for endomycorrhizal assay. Canadian Journal of Microbiology 26: 535-538.
26. Koske, R.E. and J.N. Gemma. 1989. A modified procedure for staining roots to detect VA mycorrhizas. Mycological Research 94 (4): 486-488.
27. McGonigle, T.P., M.H. Miller, D.G. Evans, G.L. Fairchild, and J.A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytologist 155: 495-501.
28. Goodall, D. W. 1957. Some considerations in the use of point quadrats for the analysis of vegetation. Australian Journal of Biological Sciences 5:1-41.
29. Packard, S., and C.F. Mutel. 1997. The Tallgrass Restoration Handbook for Prairies, Savannas, and Woodlands. Island Press, Washington D.C.

30. van der Valk, A.G., and C.B. Davis. 1978. The role of seed banks in the vegetation dynamics of prairie glacial marshes. Ecology 59: 322-335.

31. Keever, C. 1950. Causes of succession on old fields of the Piedmont, N.C. Ecological Monographs 20: 229-250.

32. Marschner, H., and B. Dell. 1994. Nutrient uptake in mycorrhizal symbiosis. Plant and Soil. 159: 89-102.

33. Sylvia, D.M., L.C. Hammond, J.M. Bennett, J.H. Haas, and S.B. Linda. 1993. Field response of maize to a VAM fungus and water management. Agronomy Journal 85: 193-198.

34. Perrin, R. 1990. Interactions between mycorrhiza and diseased caused by soil-borne fungi. Soil Use Management 6: 189-195.

35. Kahn, A.G. 1974. The occurrence of mycorrhizas in halophytes, hydrophytes and xerophytes and of Endogone spores in adjacent soils. Journal of Genetics and Microbiology 81: 7-14.

36. Gerdemann, J.W. 1974. Mycorrhizae. Pp 205-217 in E.W. Carson (ed.) The Plant Root and Its Environment: Part 1. Virginia Polytechnic Institute and State University, Charlottesville,.

37. Secilia, J., and D.J. Bagyaraj. 1992. Selection of an efficient vesicular-arbuscular mycorrhizal fungi for wetland rice (*Oryza sativa* L.). Biology and Fertility of Soils 13: 108-111.

38. Anderson, R.C., A.E. Liberta, and L.A. Dickman. 1984. Interaction of vascular plants and vesicular-arbuscular mycorrhizal fungi across a soil moisture gradient. Oecologia 64: 111-117.

39. Rickerl, D.H., F.O. Sancho, and S. Ananth. 1994. Vesicular-arbuscular endomycorrhizal colonization of wetland plants. Journal of Environmental Quality 23: 913-916.

40. Wetzel, P.R., and A.G. van der Valk. 1996. Vesicular-arbuscular mycorrhizae in prairie pothole wetland vegetation in Iowa and North Dakota. Canadian Journal of Botany 74: 883-890.

41. Liberta, A.E., R.C. Anderson, and L.A. Dickman. 1983. Vesicular-arbuscular mycorrhiza fragments as a means of endophyte identification at hydrophytic sites. Mycologia 75: 169-171.
42. Tanner, C.C., and J.S. Clayton. 1985. Effects of vesicular-arbuscular mycorrhizas on growth and nutrition of a submerged aquatic plant. Aquatic Botany 22: 377-386.
43. Khan, A.G. 1993. Occurrence and importance of mycorrhizae in aquatic trees of New South Wales, Australia. Mycorrhiza 3: 31-38.
44. Mejstrik, V. 1965. Study of the development of endotrophic mycorrhiza in the association of Cladietum marisci. Pp 283-290 in J. Macur, V. Vencura (eds). Plant microbes relationships. Proceedings of the Symposium on Relationships Between Soil Microorganisms and Plant Roots. Publishing House of the Czechoslovak Academy of Science, Prague,
45. Mejstrik, V. 1976. The ecology of mycorrhiza in plants from peat bog areas of the Trebon Basin in relation to the ground water table. Quaest. Geobiol. 16: 99-174.
46. Chaubal, R., G.D. Sharma, and R.R. Mishra. 1982. Vesicular arbuscular mycorrhiza in subtropical aquatic and marshy plant communities. Proceedings of the Indian Academic Society of Plant Science 91: 69-77.
47. Clayton, J.S., and D.J. Bagyaraj. 1984. Vesicular-arbuscular mycorrhizas in submerged aquatic plants of New Zealand. Aquatic Botany 19:251-262.
48. Maynard, D.G., and Y.P. Kalra. 1993. Nitrate and exchangeable ammonium nitrogen. Pp 25-38 *in* M.R. Carter (ed.) Soil Sampling and Methods of Analysis. Canadian Society of Soil Science, Lewis Publishers.
49. Dhillion, S.S. 1993. Vesicular-arbuscular mycorrhizas of *Equisetum* species in Norway and the U.S.A. : occurrence and mycotrophy. Mycological Research 97: 656-660.
50. Hayman, D.S. 1983. The physiology of vesicular-arbuscular endomycorrhizal symbiosis. Canadian Journal of Botany 61: 944-963.
51. Mortimer, C.H. 1941. The exchange of dissolved substances between mud and water in lakes. I-II. Journal of Ecology 29: 280-329.

52. Mortimer, C.H. 1942. The exchange of dissolved substances between mud and water in lakes. III-IV. Journal of Ecology 30: 147-201.

53. Gambrell, R.P., and W.H. Patrick Jr. 1978. Chemical and microbiological properties of anaerobic soils and sediments. Pp 375-423 *in* D.D. Hook and R.M.M. Crawford (eds.) Plant Life in Anaerobic Environments. Ann Arbor Press, Ann Arbor, MI.

54. Chambers, R.M., and W.E. Odum. 1990. Porewater oxidation, dissolved phosphate and the iron curtain: Iron-phosphorus relations in tidal freshwater marshes. Biogeochemistry 2.

55. Bergmann, W. 1992. Nutritional disorders of plants - development, visual and analytical diagnosis. Fischer-Verlag, Jena.

56. McJannet, C.L., P.A. Keddy and F.R. Pick. 1995. Nitrogen and phosphorus tissue concentrations in 41 wetland plants: a comparison across habitats and functional groups. Functional Ecology 9: 231-238.

57. Ponnamperuma, F.N. 1972. The chemistry of submerged soils. Advances in Agronomy 24: 29-96

58. Johansen, A., I. Jakobsen, and E.S. Jensen. 1992. Hyphal transport of 15N-labelled nitrogen by a vesicular-arbuscular mycorrhizal fungi and its effect on depletion of inorganic soil N. New Phytologist 122: 281-288.

59. Smith, S.E., B.J. St. John, F.A. Smith, and D.J.D. Nicholas. 1985. Activity of glutamine synthetase and glutamate dehydrogenase in *Trifolium subterraneim* L. and *Allium cepa* L.: effects of mycorrhizal infection and phosphate nutrition. New Phytologist 99: 211-227.

60. Law, R., and D. H. Lewis. 1983. Biotic environments and the maintenance of sex - some evidence from mutualistic symbioses. Biological Journal of the Linnean Society 20: 249-276.

61. Heywood, V. H. 1993. Flowering Plants of the World, 2nd edition. Oxford University Press, New York. 335 pages.
62. Allen, E. B., M. F. Allen, D. J. Helm, J. M. Trappe, R. Molina, and E. Rincon. 1995. Patterns and regulation of mycorrhizal plant and fungal diversity. Plant and Soil 170: 47-62.
63. Zobel, M., M. Moora, and E. Haukioja. 1997. Plant coexistence in the interactive environment: arbuscular mycorrhiza should not be out of mind. Oikos 78: 202-208.
64. Bever, J.D., J.B. Morton, J. Antonovics, and P.A. Schultz. 1996. Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. Journal of Ecology 84: 71-82.
65. Morton, J.B., S.P. Bentivenga, and W.W. Wheeler. 1993. Germ plasm in the International Collection of Arbuscular and Vesicular-arbuscular Mycorrhizal Fungi (INVAM) and procedures for culture development, documentation and storage. Mycotaxon 48: 491-528.
66. Morton, J.B., and E. Thomas, directors. International Culture Collection of Arbuscular and Vesicular Arbuscular Mycorrhizal Fungi (INVAM) web site: invam.caf.wvu.edu
67. Schenk, N.C., and Y. Perez. 1988. Manual for the Identification of VA Mycorrhizal Fungi, 2nd ed. INVAM, University of Florida, Gainsville.
68. Hetrick, B. A. Daniels, and J. Bloom. 1986. The influence of host plant on production and colonization ability of vesicular - arbuscular mycorrhizal spores. Mycologia 78: 32-36.
69. Millner, P.D., and D. G., Kitt. 1992. The Beltsville method for soilless production of vesicular-arbuscular mycorrhizal fungi. Mycorrhiza 2: 9-15.
70. Hetrick, B.A.D., D.G. Kitt, and G.T. Wilson. 1988. Mycorrhizal dependence and growth habit of warm-season and cool-season tallgrass prairie plants. Canadian Journal of Botany 66: 1376-1380.

71. Hoagland, D.R., and W.C. Snyder. 1933. Nutrition of strawberry plant under controlled conditions: (a) effects of deficiencies of boron and certain other elements; (b) susceptibility to injure from sodium salts. Proceedings of the American Society of Horticultural Science 30: 288-294.

72. Marschner, H. 1995. Mineral Nutrition of Higher Plants, 2nd ed. Academic Press, London.