THE USE OF MYCORRHIZAL FUNGI IN RESTORATION OF HIGHLY DISTURBED SOILS IN BOULDER COUNTY OPEN SPACE LANDS

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Vera S. Evenson
Thomas Grant
Michelle DePrenger-Levin

Denver Botanic Gardens
Research and Conservation Department

Denver Botanic Gardens

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Project Contacts:

Vera S. Evenson
Manager of Mycology
Denver Botanic Gardens
909 York Street
Denver, CO 80206
720 865-3651
EVENSONV@botanicgardens.org

Thomas A. Grant
Manager of Research Programs
Denver Botanic Gardens
909 York Street
Denver, CO 80206
720 865-3562
GRANTT@botanicgardens.org

Michelle DePrenger-Levin
Research Assistant
Denver Botanic Gardens
909 York Street
Denver, CO 80206
720 865-3630
DePrengM@botanicgardens.org

ABSTRACT

The goal of this study was to explore the value of using different kinds of mycorrhizal inoculum (commercially available and native soil-borne fungi) for determining the best methods for restoring a degraded road within the Heil Ranch Open Space, a Boulder County Parks and Open Space property. A primary goal was to test practical methods that Boulder County could potentially use on other restoration projects. The study site was an abandoned road overgrown with weedy non-native plants, prior to a spring prescribed burn and seeding of native grasses. Control, commercial inoculum, and native soil inoculum treatments were randomly located within the roadbed and samples were collected for the analysis of vegetation cover, biomass, and species composition. Statistically significant results were revealed between plots for both cover and biomass, although overall treatments were not significantly different. The data show similar percent cover for all treatments, but much greater biomass production for the native soil and commercial inoculums. Plant species composition was dominated by weedy, non-native plants in all plots, although two native species (*Artemisia ludoviciana* and *Sporobolus cryptandrus*) ranked within the top five most prevalent species for the native soil inoculum and control treatments. A large amount of variation was documented within treatments for both percent vegetation cover and biomass production. This could be related to environmental factors (low precipitation) or site differences. Germination of seeded material may have been negatively affected by the lack of available moisture, especially since none of the seeded species were dominant in any of the plots. Additional monitoring and sampling of the plots will more accurately determine the long-term affects of the native grass seeding and experimental treatments, especially since 2002 had extremely low precipitation. Overall, the initial results determined that using native soil in the restoration of disturbed soils might facilitate greater biomass production and native species composition than a commercial inoculum or control treatments.

INTRODUCTION

Vesicular arbuscular mycorrhizal fungi (VAM) have been shown to play an important role in the restoration of disturbed and degraded soils and ecosystems (Reeves et. al. 1979; Allen 1991; Read et. al. 1992; Wilson et. al. 2001). This research has shown that among the benefits to plants from root infection and colonization by VAM are improved uptake of nutrients, principally phosphate, but also ions such as copper, zinc, chloride, sulfate and ammonium. In addition, VAM provide considerable protection from root pathogens as well as giving them the ability to withstand higher soil temperatures, higher soil salinity, and wider extremes of soil pH (Palm and Chapela 1997). The USDA has demonstrated that VAM produce Glomalin, a compound that increases soil aggregation and infiltration, as well as water-holding capacity. Similar fungi have been termed Arbuscular Mycorrhizal Fungi (AMF), instead of Vesicular Arbuscular Mycorrhiza (VAM) and therefore some disagreement exists in determining the appropriate name. The controversy has persisted because not all endomycorrhizal fungi produce vesicles and arbusculars are not always present (CSIRO 2002). Throughout this paper all endomycorrhizal fungi will be termed VAM. In the spring of 2002, an experimental road restoration project was initiated in the Geer Canyon of Heil Ranch Open Space as part of the Boulder County Open Space Small Grants Program. Using techniques that could be applied to larger scale habitat restoration projects, Denver Botanic Gardens tested the effects of commercial mycorrhizae and native soil inoculums on the vegetative cover and biomass production of a mountain meadow following a prescribed burning and broadcast seeding of native species within a degraded roadbed.

In natural undisturbed ecosystems, propagules of VAM are concentrated in the uppermost few centimeters of soil. When the upper portion of the soil is damaged by compaction, degradation, non-native weed invasion, or other detrimental effects, the propagules of natural soil fungi are greatly reduced in numbers and viability. Reintroduction of VAM may hold an answer for the restoration of that natural ecosystem (Tallaksen, 1996). This project sought to examine the effects of introducing VAM to disturbed and compacted soils of an old road in need of restoration by comparing the introduction of a commercial inoculum containing VAM with the introduction of locally collected top soil potentially containing native VAM.

The research site selected for the study was in an essentially tree-less meadow in Geer Canyon on Heil Valley Ranch owned by Boulder County. In the open mountain meadow gently sloping to the east, the presence of an old road resulted in the compaction and degradation of the site, leaving an unattractive and weed infested "scar". The roadbed had been covered with a coarse fill that became overgrown with several types of non-indigenous plants, thus restricting growth of native species. As far as could be determined, the area chosen for the study plots was more or less uniform in its vegetative cover and species diversity.

The project consisted of four phases: 1) Choosing a project location and marking out experimental and control plots; 2) Introducing two types of inoculum (commericial and native soil) into the randomly selected plots directly after the prescribed burning and sowing of a standard seed mix; 3) Monitoring the site for changes in vegetation for a period of 5 months and photographing the cover in each plot; 4) Harvesting 10 randomly selected subplots within each plot in order to assess vegetation cover, biomass, species diversity, and VAM infection success. The experimental project was initiated with the purpose of testing potential restoration methods that could be applied by Boulder County Open Space to existing natural areas. The primary goal is to evaluate the effectiveness of commercially available mycorrhizal inoculants and native soil inoculants on the restoration of the degraded roadbed using percent vegetation cover, biomass, and diversity of species as the quantitative measurements.

MATERIALS AND METHODS

Originally, the degraded roadbed test site was covered by a relatively dense growth of non-indigenous, weedy plants and a few native grasses and forbs. The field experiment began in late March, 2002 by establishing six study plots of 9 square meters, each within the old roadbed. A narrow walkway space between each plot was established in order to allow monitoring and photography. Following our marking of the six test plots, Boulder County employees conducted a prescribed burn of the entire meadow in early April, 2002. After the fire we noted that some non-native species, particularly *Anisantha tectorum* (Cheatgrass) and *Verbascum thapsum* (Great Mullein), were not completely burned and some living, aboveground growth could be identified within the roadbed. Boulder County then seeded the old roadbed area, including our marked plots, with a seed mix commonly used in their revegetation programs. The prescribed burn affected most of the meadow and all of the former road, but only the road was seeded. Appendix A lists the plant species included in the seed mix and application rates. On April 19, 2002, immediately after the broadcast seeding, our research team treated two plots with AM120 commercial inoculum and two plots with a native soil inoculum.

The application of the treatments and controls were randomly applied to the six plots along the roadbed. Appendix B illustrates the experimental design for the restoration project and lists the treatments applied to each plot. The two experimental treatments were carried out as follows: the commercially available VAM inoculum product (AM120 Reforestation

Technologies International, purchased from Pawnee Buttes Seed Company of Greeley, CO) consisting of propagules of three VAM species of *Glomus (G. intraradices, G. aggregatum, G. mosseae)* was broadcast at the recommended application rate (0.7kg per 100m^2) for the two experimental plots (3 and 6) and gently raked into the soil surface. An additional application of VAM inoculum was applied to the soil surface, therefore the recommended rate was doubled although half of the inoculants were mixed with the topsoil. Native soil inoculum was obtained locally from topsoil set aside during construction of a vault toilet at the Heil Ranch Open Space. Soil material exposed to sunlight or near the surface of the storage pile was not collected, due to possible contamination by weed seeds or destruction of mycorrhizal fungi from direct solar radiation or temperature extremes. Approximately three gallons of the topsoil were broadcast evenly over each of the two experimental plots (4 and 5) and then gently raked. The control plots were not inoculated or manipulated, although they received the same burning and seeding treatments as the experimentally plots. An additional control plot was installed within the roadbed on August 13, 2002. The seventh plot was added to aid in the assessment of control plots, since the two original control plots had been randomly assigned to the top of the meadow near the current road, which had a gentler slope and appeared to be more disturbed.

From April through September 2002, Denver Botanic Gardens researchers visited and photographed the test plots every two to three weeks. It was noted on several visits that the severe drought conditions were limiting growth of the vegetation (Appendix D). Because of the drought conditions, we decided to delay collecting of the samples for biomass and percentage cover studies with the hope that late summer rains would promote additional growth. Collecting of biomass was done on September 26, 2002 after several rains storms had stimulated growth of vegetation within the meadow.

Within the seven test plots, potential edge effects were reduced by creating 2m x 2m plots in the center of each 3m x 3m plot for the sampling of cover and biomass. Percent vegetation cover and biomass were sampled within each plot using ten randomly placed 20cm x 20cm frames (Appendix C). If sampling frames overlapped with a previous sample, new random coordinates were generated. Overall, 10% of each 2m x 2m plot was sampled and a total of 10 samples were taken from each of the seven plots. A grid placed over the sampling frames for the analysis of basal vegetation cover divided the sample area into 25 quadrats (each 4cm X 4cm), each consisting of 4% of the total area. Using small areas for the estimation of cover reduces variability between observers and increases reliability of the sampling method (Elzinga et. al., 1998). We counted each 4cm X 4cm quadrat with greater than 50% cover and determined the percent cover for the sample based on the number of 'covered' versus 'uncovered' quadrats (Tables 1 and 2). After cover was estimated, all of the biomass (living and dead) was cut to ground level in each of the 20cm x 20cm sample frames and collected for laboratory analysis. Biomass quantifies the amount of annual production by harvesting all aboveground standing vegetation. Only biomass that originates basally within the sample frame was collected for the study. Dead biomass (last year's growth) was removed from the current year's growth, although little biomass from previous years remained after the prescribed burn. The collected biomass was dried at 32 to 35 degrees Celsius until no additional weight loss from the evaporation of water could be detected. Samples were dried for seven days before the final weighing of biomass (dry weight in grams) and identification of the three dominant plant species within each sample. The mean percent cover and biomass (grams) were calculated for each plot (n = 10) and each treatment (n = 20 for commercial and native soil inoculants, n = 30 for control). Tables 3 and 4 display the mean biomass per plots and treatments, respectively.

A method of ranking the dominant species within each sample was developed based on scoring the three species which dominated the biomass for each of the 70 samples. The purpose was to qualitatively determine the predominant species for each of the plots and treatments. Due to the drought and the dominance of small plants (example – many cheatgrass seedlings were less than 2cm tall), it was impossible to separate individual species for weighing. Therefore, the three dominant species were determined visually during the weighing of samples, based on proportional volume of sample, plant size, and number of plants. The dominant species for each sample received a score of 3, the second dominant scored a 2, and the third dominant species scored a 1. The scores for all species included in the plot were tabulated and the dominant species ranked in descending order of prevalence for each treatment (Table 5).

In order to assess the amount of mycorrhizal fungi present between different plots and treatments, four species of plants were collected from each plot. Microscopic study of mycorrhizal colonization were done by carefully removing four species in each study plot to a depth of at least 10 cm. All soil was washed off of the roots, care being taken to preserve attachment of fine terminal feeder roots. Lengths of the small roots were then cut off and stored in a fixative F.A.A. (90 % formalin, 5% acetic acid, 5% ethanol). Microscopic staining with lacto-phenol trypan blue and observation for arbuscules and vesicles are currently being carried out to qualitatively assess the success of mycorrhizal colonization for each of the four plant species from the different treatments and plots. Methods used for these studies are those of T. P. McGonigle et. al. (1990), R. B. Mullen and S.K. Schmidt (l993). This aspect of the project was a small portion of the original grant proposal, but has grown into a quantitative study and deemed necessary to contrast the amount of VAM fungi within each of the treatments.

RESULTS

The mean percent vegetation cover was calculated for each plot (n = 10) and summarized in Table 1. The results range from a high of 68.4% vegetation cover for plot 5 (native soil inoculant) to a low of 24.8% for plot 6 (commercial inoculant). A one-way analysis of variance (ANOVA) compared the percent cover between plots and found statistically significant differences between plots ($P < 0.001$, n = 70). Table 2 illustrates the mean percent vegetation cover for each treatment. The control treatment had the highest mean percent cover (52.5%), although the range between the treatments was narrow (7.9%). A similar statistical test compared percent cover and treatment, but the results were not significant ($P = 0.569$, n = 70).

Biomass represents the amount (grams) of vegetation produced within each sample during the 2002 growing season. Table 3 illustrates the mean biomass per plot and dominant species based on the ten samples collected within each of the seven plots. The native soil inoculant (plot 5) has the highest mean biomass (12.05 grams), followed by plot 3 (9.29 g, commercial inoculum), then plot 4 (7.8 g, native soil inoculant). The second commercial inoculant (plot 6) had the lowest mean biomass production (2.86 g). A one-way analysis of variance (ANOVA) determined statistical significance between plots for biomass production ($P = 0.001$, n = 70). Using a Tukey's post hoc test of multiple comparisons, the following contrasts (statistical comparisons between plots) were determined to statistically differ at alpha = 0.05:

Plots	Treatments
3:4	Commercial vs. Native
3:6	Commercial vs. Commercial
4:5	Native vs. Native
5:6	Native vs. Commercial

Plots not listed in the previous table were not statistically significant with any other plots. Plot 5 has the greatest mean biomass and was the only plot in which the dominant species was a native, desirable plant (*Sporobolus cryptandrus*, Sand Dropseed).

Table 4 lists the mean biomass and dominant species for each treatment. Overall, the native soil treatment had the highest mean biomass (9.925 g, n = 20) and the control treatment had the lowest mean biomass (5.09 g, n = 30). A similar statistical test comparing the biomass production between treatments was not significant (P = 0.976, n = 70). The overall dominant species for each of the treatments was non-native.

The results of the ranking system determined the dominant plant species for each plot and treatment based on the 10 samples collected from each plot. Table 5 lists the dominant species and corresponding scores for each plot and summarizes the overall scores for each treatment. In general, Cheatgrass (*Anisantha tectorum*), Bindweed (*Convolvulus arvensis*), and Crane's Bill (*Erodium cicutarium*) were the dominant species in most of the plots and treatments. There are several notably exceptions. In the control plots, *Artemisia ludoviciana* (Prairie Sage) was the third most dominant species and *Sporobolus cryptandrus* (Sand Dropseed) was the fifth most prevalent species. The native soil treatment was dominated by Cheatgrass and Crane's Bill, but the third most dominant species was *Sporobolus cryptandrus*, a native grass, and the forth most prevalent species was *Artemisia ludoviciana*. The native grass species was not part of the seed mix and therefore could have been introduced to the site within the native soil treatment, although it was also found in the control plots.

Weather data for a Boulder, Colorado weather station (# 050848) was collected from the Western Regional Climate Center in Reno, Nevada (WRCC 2002). Based on measurements from 1948 thru 2001, this weather station has a mean annual precipitation of 19.11 inches. In 2002, a total of 13.47 inches of precipitation were received. Although no data was available for December, this month normally receives little precipitation (0.7 inches). Appendix D lists the mean and 2002 precipitation data for the Boulder weather station. Three extremely dry months occurred during the growing season and project timeline: April, June, and July. Each of these months was dramatically below the mean monthly precipitation based on the 53 years of measurement. April received 2.21 inches below the mean, June was 0.99 inches below, and July was 1.78 inches below the monthly mean amount of precipitation.

The microscopic analysis of root specimens of four species from each plot are currently being analyzed by Vera Evenson. The laboratory work could not be completed in time for the report due to difficulty obtaining the necessary staining chemicals and determining the correct methodology. This aspect of the project has been expanded beyond the original plan mentioned in the proposal, due to its importance for quantifying differences in mycorrhizal levels in the three treatments and four species sampled. The microscopy will be completed during the winter of 2003 and submitted to Boulder County Parks and Open Space as an addendum to the final report.

DISCUSSION

The restoration of natural ecosystems is an essential component in the management of natural resources, especially when open spaces are increasingly surrounded and influenced by development, degraded habitats, and recreational pressures. In order to sustain natural areas in perpetuity, ecologically sensitive management practices are necessary to support the naturally evolved and dynamic systems. At the Heil Ranch, Boulder County Parks and Open Space has initiated programs of prescribed burns and seeding of native species to assist in the restoration of a meadow that has been degraded by an old road. Denver Botanic Gardens has added an experimental aspect to the restoration of this roadbed restoration in Geer Canyon. Using commercially available mycorrhizal fungi and native soil-borne mycorrhizae, we quantitatively and qualitatively tested the effects on vegetation cover, biomass production, and species composition. By adding an experimental component into a restoration project, it is possible to determine methods or techniques that can be beneficial to the goals of an ecological restoration. A primary goal for this project was to test practical methods that Boulder County could potentially use in future restoration projects.

The summer of 2002 was a drought year and this undoubtedly affected the growth of existing vegetation and the germination of seeded material and mycorrhizae (Appendix D). Following the burning and seeding of the mountain meadow, we saw little growth of vegetation until late August. Originally, we had planned to collect the biomass and cover data in August, but this was delayed to allow more time for the vegetation to grow. Unfortunately, we saw large amounts of *Anisantha tectorum* (Cheatgrass) seedlings emerging from the ground following the late summer rains. Cheatgrass is an annual (or winter annual) species that is non-native and considered to be a fire hazard and harmful to animals because of the spiked awns, which harm the mouths of grazers (Harrington, 1954; Weber and Wittman, 2001). The prescribed burn may not have been intense enough to kill the soil seed bank of Cheatgrass propagules, possibly due to insufficient vegetation or litter along the road to sustain the fire. Biomass samples were collected between September 26 and October 4 2002, although vegetative growth occurred throughout October at the study site (Evenson, Personal Observation).

The vegetation cover data estimates the amount of cover within the study sites. Cover can affect many factors of a microsite, including: solar insulation, soil temperature, erosion, plant competition, germination, and recruitment. Areas of high percent cover are less likely to have soil erosion and may be less susceptible to invasion by non-native, weedy species. Unfortunately, many of the dominant species within the roadbed are established non-natives. The mean percent cover by plot (Table 1) illustrates the large variation in cover between plots. The ANOVA results highlight statistically significant variation between plots, but not between treatments. Therefore, we acknowledge that the plots varied considerable, but the treatments could not be shown to differ statistically from each other, possibly due to the great variation in the individual samples. Overall, the control had the highest percent cover, but the difference between the control and native soil treatments were minimal, a 0.73% difference (Table 2). Overall, no consistent variation in percent vegetation cover could be identified for the experimental treatments. Assuming greater precipitation in 2003, it may be possible to recognize the affects of the treatments on the vegetation cover.

Biomass production data augment the information gathered from cover, especially when many predominant species are graminoids. Grasses tend to cause an over estimation of basal cover and add little weight to biomass samples, while a single native prairie sage (*Artemisia ludoviciana*) or invasive musk thistle (*Carduus nutans*) may add little basal cover, but lots of biomass. Therefore, differences between cover and biomass should be expected. The highest producing plots were native soil (plot 5), commercial (3), and native soil (4), respectively. The three control plots ranked forth, fifth, and sixth in biomass production and the remaining commercial inoculant (plot 6) produced the least biomass (Table 3). Plot 6 probably represents an outlier due to unknown circumstances that limited vegetation growth, possibly greater soil compaction or erosion than the other plots. The ANOVA did determine that the plots were statistically significant from each other (see Tukey test in results), although the differences only occurred between the plots with the two experimental treatments applied (commercial inoculant and native soil inoculant). The control plots did not differ statistically from any of the experimental treatments. Overall, treatments were not shown to differ statistically from each other, possibly due to the large variation between samples. On average, the native soil inoculants produced the most biomass, followed by the commercial, then the control treatments (Table 4). Although the data does not represent clear statistical differences between treatments, the summary information in Tables 3 and 4 illustrates the greatest biomass production in the native soil and commercial treatments.

The data lead us to believe that native soil had the greatest effect on the production of biomass and species composition. Two of the top five dominant species in the native soil plots were native (Table 5). These taxa (*Artemisia ludoviciana* and *Sporobolus cryptandrus*) are also present in the control plot rankings, but at a lower level and may be an indication of plant species that are able to compete with the non-natives, primarily Cheatgrass, Bindweed, and Crane's Bill. These are good results compared to the species composition of the commercial and control plots. Drought conditions likely stifled germination of the seeded materials, therefore the actual results of this experiment may not be revealed for several years. The mycorrhizal spores and seeded material will probably remain viable for several years, therefore additional sampling of the seven plots is suggested in order to determine the outcome of the experiment. In summary, the experimental treatments did not appear to effect the vegetation cover, but biomass production seemed to increase in the two experimental treatments, possibly due to the introduction of mycorrhizal fungi. Additionally, the prevalence of native species was greater in the native soil inoculant treatment, possibly due to the introduction of native propagules within the soil or the suppression of existing weeds due to the addition of soil to the site. Sampling of the study sites in an average precipitation year and the results of the microscopic study quantifying VAM infection in the different treatments, will provide additional data to determine the results of this experiment. The use of mycorrhizal inoculants in restoration projects is a fairly new practice, especially in experimental situations in natural environments. The initial results are not simple to interpret, but in time and with patience we can determine the results of the experiment and the appropriate methods for restoring degraded roads along the mountains of Colorado's Front Range.

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Table 1 – Mean Percent Vegetation Cover by Plot
Data arranged in descending order by percent cover, sample size (n) = 10 for each plot.

Plot	Treatment	Mean % Cover
5	Native Soil Inoculant	68.4
1	Control	64.4
3	Commercial Inoculant	64.4
7	Control	52
2	Control	41.2
4	Native Soil Inoculant	35.2
6	Commercial Inoculant	24.8

Table 2 – Mean Percent Vegetation Cover by Treatment
Data arranged in descending order by percent cover.

Treatment	Mean % Cover	N
Control	52.53333333	30
Native Soil Inoculant	51.8	20
Commercial Inoculant	44.6	20

Table 3 – Mean Biomass Production by Plot

Data arranged in descending order by biomass, sample size (n) = 10 for each plot. Dominant species determined by ranking system based on visual estimate of the three dominant species in each biomass sample (see methods).

Plot	Treatment	Mean Biomass weight (grams)	Dominant Species	Nativity of Dominant
5	Native Soil Inoculant	12.05	Sporobolus cryptandrus (Torr.) Gray	Native grass (Sand Dropseed)
3	Commercial Inoculant	9.29	Anisantha tectorum (L.) Nevski	Non-native (Cheatgrass)
4	Native Soil Inoculant	7.8	Erodium cicutarium (L.) L'Hér. ex Ait.	Native, weedy & ruderal (Crane's bill)
1	Control	5.6	Anisantha tectorum (L.) Nevski	Non-native (Cheatgrass)
7	Control	5.55	Convolvulus arvensis L.	Non-native (bindweed)
2	Control	4.14	Anisantha tectorum (L.) Nevski	Non-native (Cheatgrass)
6	Commercial Inoculant	2.86	Convolvulus arvensis L.	Non-native (bindweed)

Table 4 – Mean Biomass Production by Treatment

Treatments arranged in descending order by biomass. Dominant species determined by ranking system of three dominant species from all samples for each treatment (see methods).

Treatment	Mean Biomass weight (grams)	N	Dominant Species	Nativity of Species
Native Soil Inoculant	9.925	20	Anisantha tectorum (L.) Nevski	Non-native (Cheatgrass)
Commercial Inoculant	6.075	20	Convolvulus arvensis L.	Non-native (Bindweed)
Control	5.096666667	30	Anisantha tectorum (L.) Nevski	Non-native (Cheatgrass)

Table 5 – Results of ranking system for dominant plant species by Treatment
Species are listed in descending order of dominance based on the combined score for all plots within the respective treatment.

Rank	Control Plots	Plot 1	Plot 2	Plot 7	Total
1 -	*Anisantha tectorum* (L.) Nevski	15	13	7	**35**
2 -	*Convolvulus arvensis* L.	12		21	**33**
3 -	***Artemisia ludoviciana* Nutt.**	10	8	9	**27**
4 -	*Bromus japonicus* Thunb. ex Murr.	7	9	2	**18**
5 -	***Sporobolus cryptandrus* (Torr.) Gray**	10	7		**17**
6 -	*Erodium cicutarium* (L.) L'Hér. ex Ait.		6	6	**12**
7 -	***Sporobolus asper* (Michx.) Kunth**	5			**5**
8 -	***Artemisia frigida* Willd.**		4		**4**
9 -	*Lactuca serriola* L.		4		**4**
10 -	*Potentilla* sp.		3		**3**
11 -	*Asteraceae* (unidentifiable)		2		**2**
12 -	***Schedonnardus paniculatus* (Nutt.) Trel.**		2		**2**
13 -	*Taraxacum officinale* G.H. Weber ex Wiggers			2	**2**
14 -	*Alyssum parviflorum* Fisch. ex Bieb.		1		**1**
15 -	*Poaceae* (unidentifiable)			1	**1**

Rank	Commercial Soil Inoculant	Plot 3	Plot 6	Total
1 -	*Convolvulus arvensis* L.		28	**28**
2 -	*Anisantha tectorum* (L.) Nevski	17	6	**23**
3 -	*Erodium cicutarium* (L.) L'Hér. ex Ait.	12	8	**20**
4 -	*Bromus japonicus* Thunb. ex Murr.	9		**9**
5 -	*Poaceae* (unidentifiable)	2	7	**9**
6 -	***Artemisia ludoviciana* Nutt.**	7		**7**
7 -	***Sporobolus asper* (Michx.) Kunth**	7		**7**
8 -	*Carduus nutans* L.	3		**3**
9 -	*Asteraceae* (unidentifiable)	2		**2**
10 -	*Trifolium* sp.		2	**2**

Rank	Native Soil Inoculant	Plot 4	Plot 5	Total
1 -	*Anisantha tectorum* (L.) Nevski	6	8	**14**
2 -	*Erodium cicutarium* (L.) L'Hér. ex Ait.	9	3	**12**
3 -	***Sporobolus cryptandrus* (Torr.) Gray**		12	**12**
4 -	***Artemisia ludoviciana* Nutt.**	8	3	**11**
5 -	*Bromus japonicus* Thunb. ex Murr.	4	7	**11**
6 -	*Convolvulus arvensis* L.	1	8	**9**
7 -	*Lactuca serriola* L.	8	1	**9**
8 -	***Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths**		6	**6**
9 -	***Artemisia frigida* Willd.**		5	**5**
10 -	***Sporobolus asper* (Michx.) Kunth**	5		**5**
11 -	***Buchloe dactyloides* (Nutt.) Engelm.**	3		**3**
12 -	***Lepidotheca suaveolens* Nuttall.**	3		**3**
13 -	*Asteraceae* (unidentifiable)		2	**2**
14 -	*Verbascum thapsus* L.		2	**2**
15 -	*Taraxacum officinale* G.H. Weber ex Wiggers		1	**1**

APPENDIX A - Seed Mix used at Heil Ranch, April 2002

Source: Claire DeLeo (Boulder County Open Space)

Seed originally used in 2001 for the Bass & Pelican Ponds Uplands revegetation

All seed tested Feb 2001.

35.87 Bulk lbs/acre.

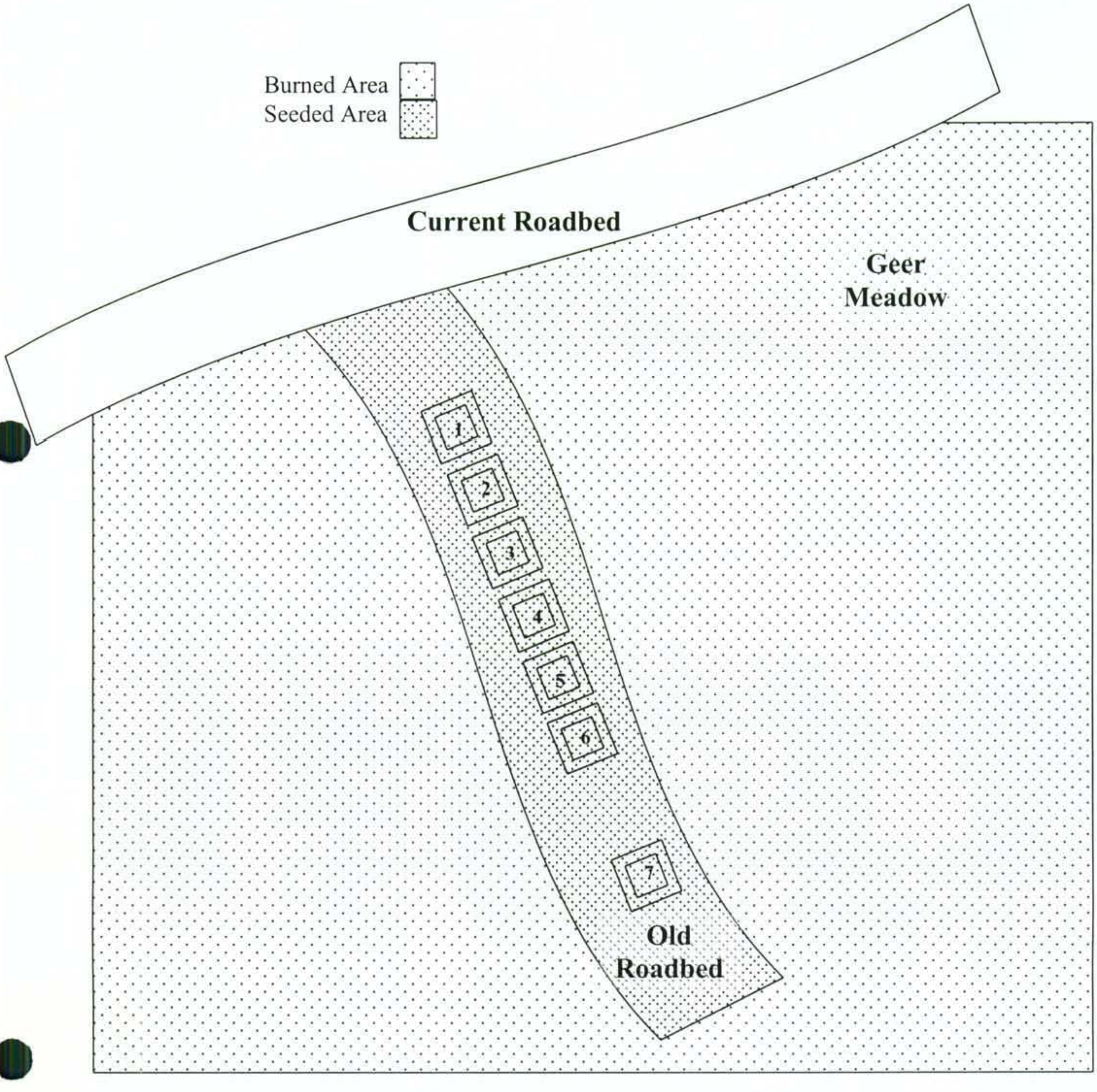
Common Name *Species* "Variety"	Approx. Seeds/Lb.	% of Mix	Total No. PLS/ft2	% of Mix #PLS/A	Origin
Side oats grama *Bouteloua curtipendula* "Vaughn"	191000	7.5	90	1.54	NM
Side oats grama *Bouteloua curtipendula* Native	191000	7.5	90	1.54	TX
Blue grama *Bouteloua gracilis* Native	825000	20	90	0.95	NM
Buffalograss *Buchloe dactyloides* Native	56000	15	90	10.50	KS
Slender wheatgrass *Elymus trachycaulus* "Revenue"	159000	10	90	2.47	Canada
Western wheatgrass *Pascropyron smithii* "Arriba"	110000	10	90	3.56	WA
Western wheatgrass *Pascropyron smithii* Native	110000	10	90	3.56	MT
Indian Ricegrass *Oryzopsis hymenoides* "Nezpar"	140000	10	90	2.80	MT

Little bluestem *Schizachyrium scoparium* "Blaze"	240000	10	90	1.63	KS
Total		**100**		**28.56**	

No. PLS/ft2 = Pounds of Pure Live Seed per square foot
#PLS/A = Pounds of Pure Live Seed per acre

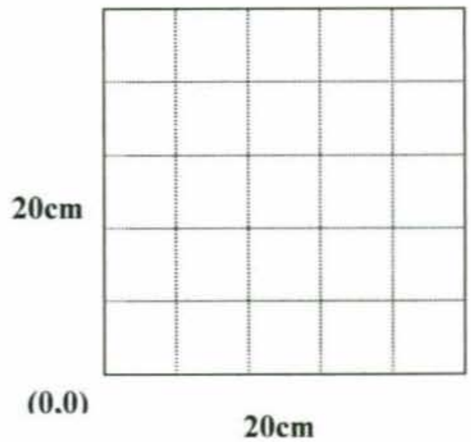
APPENDIX B - Heil Ranch Experimental Design

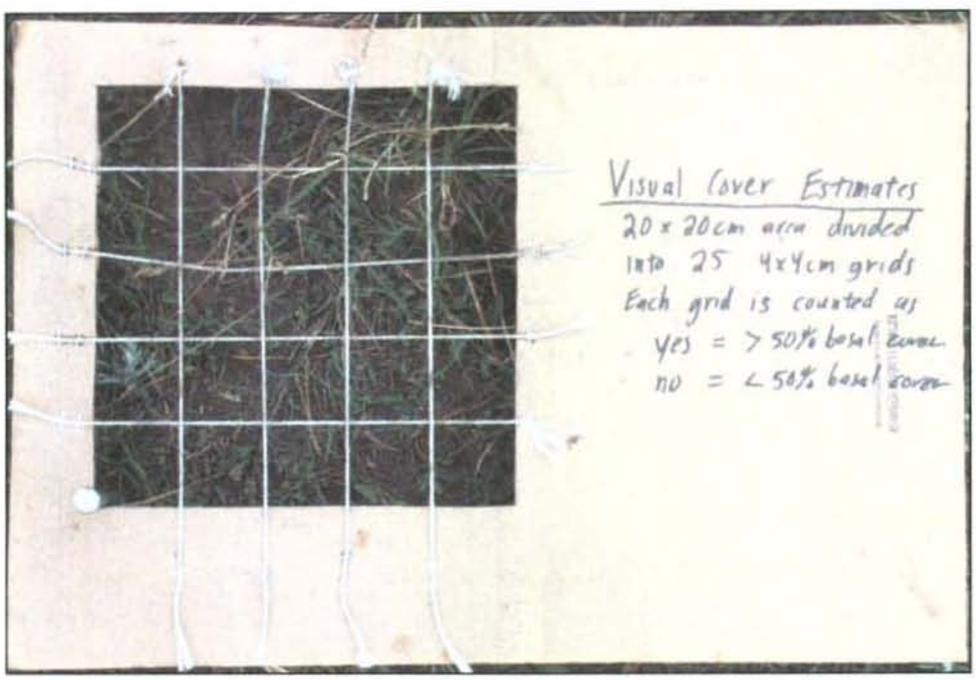
Seven experimental plots (3 x 3 meters each) were placed within the old roadbed and the following experimental treatments randomly applied: 1 = control, 2 = control, 3 = commercial VAM, 4 = native soil inoculant, 5 = native soil inoculant, 6 = commercial VAM, 7 = control. Within each plot a 2 x 2 meter sampling area was defined to reduce edge effects.



APPENDIX C – Cover and biomass sampling frames

Sampling frames were used to delineate biomass collection areas and define 25 quadrats (each 4cm X 4cm and 4% of sampling area) for estimation of percent cover. Percent cover based on methods of Elzinga et al, 1998.





APPENDIX D – Weather Data

Source: Western Regional Climate Center website, Desert Research Institute, Reno, Nevada. http://www.wrcc.dri.edu/

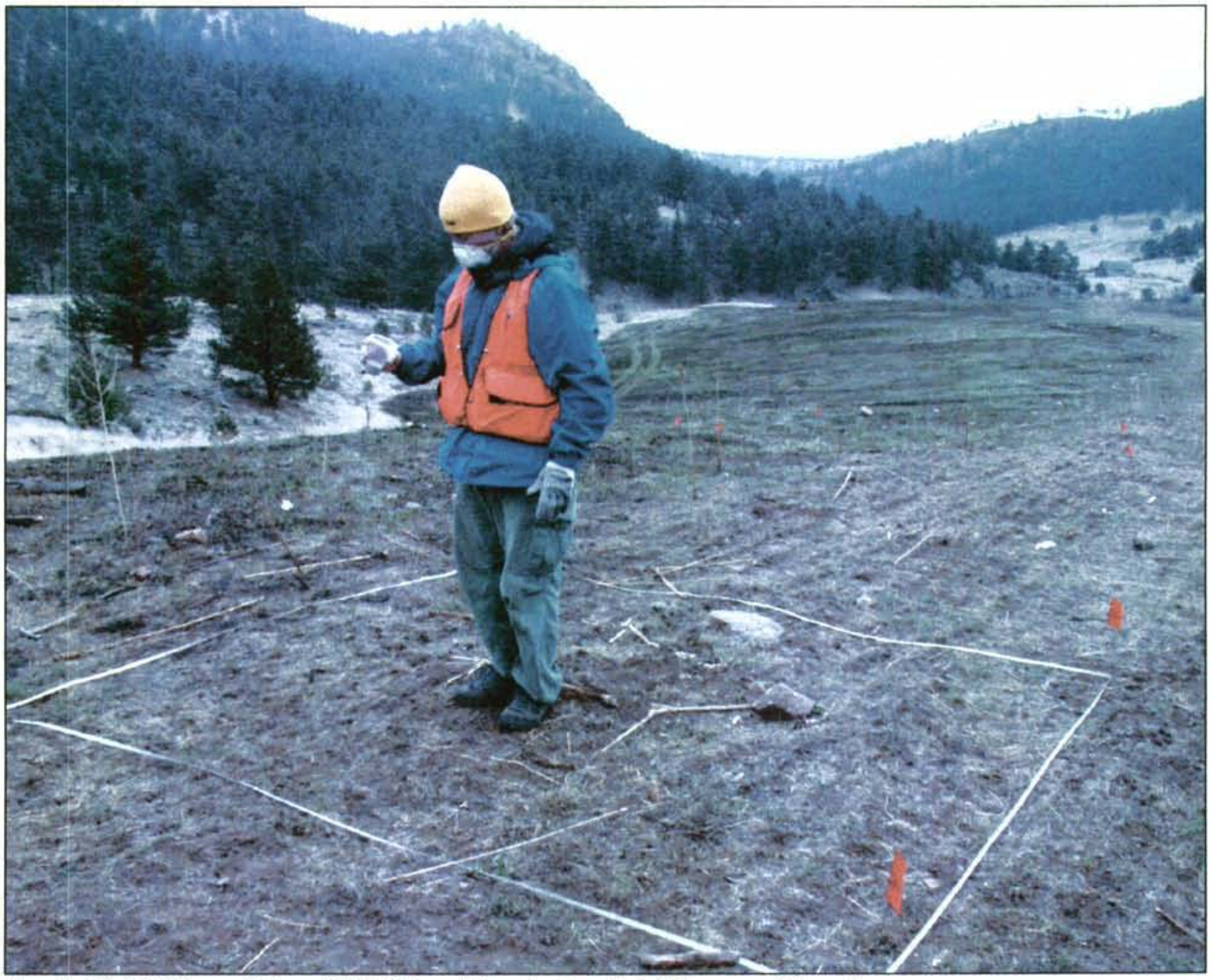
2002 Monthly Total Precipitation in Inches Boulder, Colorado (050848)												
JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	Cumulative
1.07	0.44	1.5	0.2	3.2	1.18	0.09	1.44	1.52	2.44	0.391	no data	13.471

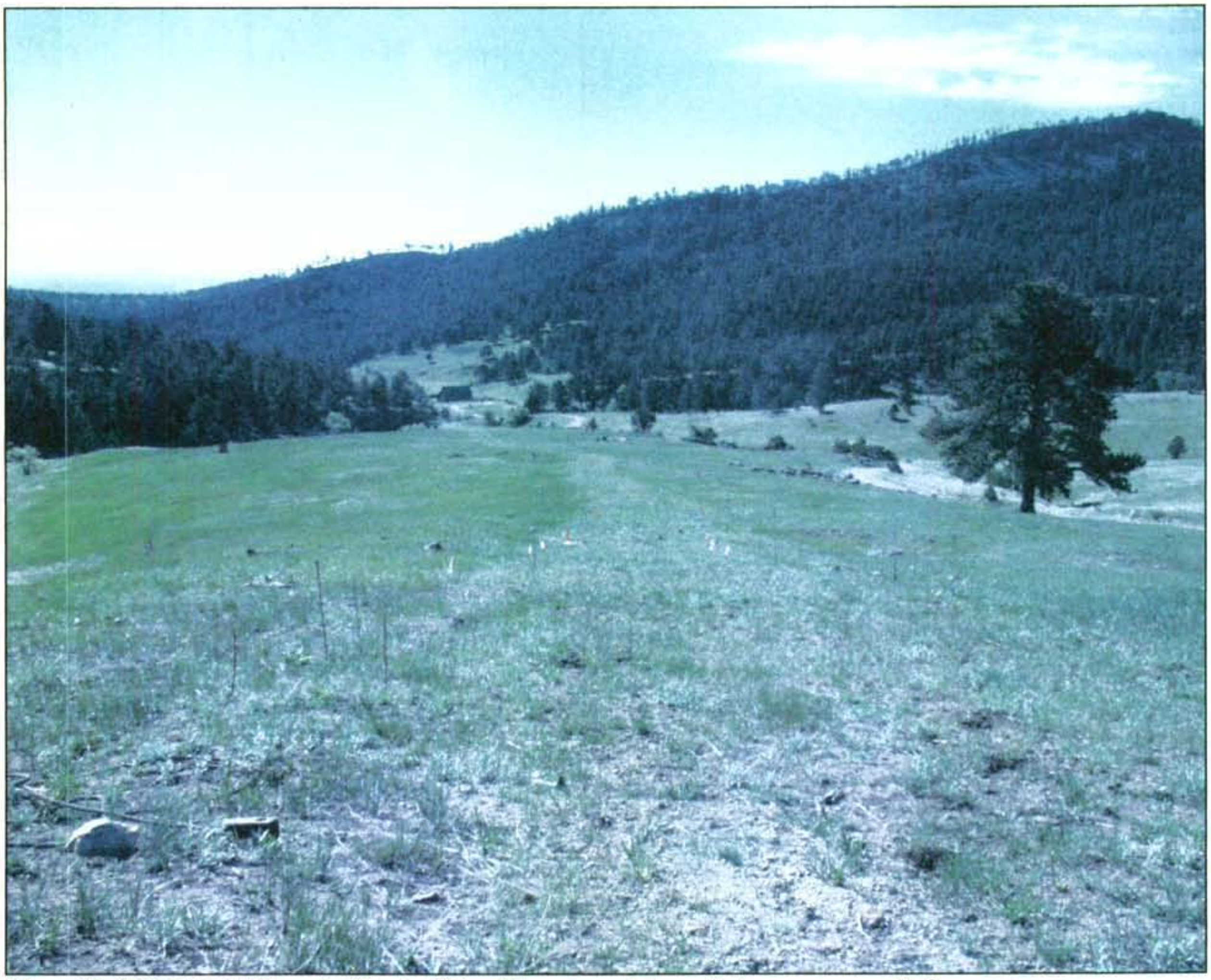
Average Total Monthly Precipitation in Inches Period of Record: 1948 - 2001 Boulder, Colorado (050848)												
JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	Cumulative
0.68	0.76	1.69	2.41	3.09	2.17	1.87	1.6	1.65	1.26	1.23	0.7	19.11

APPENDIX E – Project Photographs

April 10, 2002 Geer Meadow of Heil Ranch Open Space (Boulder County Parks and Open Space) after prescribed burn.

April 10, 2002 Heil Ranch study site. Note discoloration of the old roadbed.


April 19, 2002 applying VAM fungi to study plot (Thomas Grant).



May 30, 2002 Heil Ranch Study Site. Flags marking plots can vaguely be seen along old roadbed.

September 26, 2002 biomass collection (Karen Schoen and Vera Evenson, left to right).