



Comparing inoculum potential of vesicular-arbuscular mycorrhiza from three plant species  
by Stuart Michael Levit

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Land Rehabilitation

Montana State University

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Abstract:

Many studies completed in recent years point to the potential for increasing land reclamation success by increasing the role of vesicular-arbuscular mycorrhiza (VAM) in revegetation plans. Understanding mycorrhizal infection in areas of chemical toxicity may be particularly important to land rehabilitation because of increased plant stress. To demonstrate the effects that tailings materials have on VAM infection rates the infectivity of mycorrhiza from three grass species growing in, near, and away from tailings enriched soil were compared.

Vesicular-arbuscular mycorrhizal infected roots from 1) tufted hairgrass growing in tailings enriched alluvium, 2) redtop bentgrass growing near tailings enriched soil, and 3) smooth brome growing (away from river deposited tailings) in agricultural soil were mixed in 1:1, 1:2, 1:4, and 1:8 dilutions (root:soil by volume) with sterilized greenhouse soil. Control treatments consisted of sterilized greenhouse soil material with no root inoculum. In the greenhouse, sudangrass, acting as a plant host, was seeded into these mixtures and its roots were examined for quantitative mycorrhizal infection to assess the impact that tailings material plays on mycorrhizal infectivity in the field.

In the 1:1 and 1:8 treatments there was significantly lower infectivity between both tufted hairgrass VAM and redtop bentgrass VAM and VAM from smooth brome. In the 1:2 there were significant differences between all three grass species' VAM. In the 1:4 treatment experimental error was probably the cause of unexpectedly high infection rates of tufted hairgrass. Similar to the 1:1 and 1:8 treatments, in the 1:4 treatment there was a significant difference between the infectivity of VAM from redtop bentgrass and smooth brome. Redtop bentgrass VAM was significantly less infective than smooth brome VAM at all inoculum levels. In all four treatments there were significant differences between smooth brome VAM and the VAM from one or both of the other two species. It was concluded that there are significant differences in VAM infectivity based on the source of VAM inoculum material. Increased examination frequency for infection and a longer growing period may yield more consistent results in similar infectivity studies.

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MYCORRHIZA FROM THREE PLANT SPECIES**

by

**Stuart Michael Levit**

A thesis submitted in partial fulfillment  
of the requirements for the degree

of

**Master of Science**

in

**Land Rehabilitation**

**MONTANA STATE UNIVERSITY  
Bozeman, Montana**

**May 1989**

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of a thesis submitted by

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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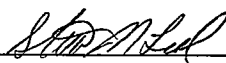
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## ACKNOWLEDGEMENTS

I wish to thank The Spokane Mushroom Club for providing financial support of this project. I would also like to thank Dr. Frank Munshower, Dr. Douglas Dollhopf, Dr. Clifford Montagne, and Mr. Dennis Neuman, all of my thesis committee, for their assistance in many aspects of planning and execution. Additional thanks go to Dr. Frank Munshower for his patience and The Reclamation Research Unit for materials and work space. Special thanks go to Bill Grey for guidance and materials throughout the project, Dr. Sharon Eversman for advice, enthusiasm, and trusting me with her microscopes, Dave Baumbauer and Perry Hofferber for greenhouse space and assistance, Mike Cormier and Chuck Hardy for analytical assistance, and my family for their support and tolerance. Finally, I would like to thank Erich Beagle for providing inspiration and direction that will last a lifetime.

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## ABSTRACT

Many studies completed in recent years point to the potential for increasing land reclamation success by increasing the role of vesicular-arbuscular mycorrhiza (VAM) in revegetation plans. Understanding mycorrhizal infection in areas of chemical toxicity may be particularly important to land rehabilitation because of increased plant stress. To demonstrate the effects that tailings materials have on VAM infection rates the infectivity of mycorrhiza from three grass species growing in, near, and away from tailings enriched soil were compared.

Vesicular-arbuscular mycorrhizal infected roots from 1) tufted hairgrass growing in tailings enriched alluvium, 2) redtop bentgrass growing near tailings enriched soil, and 3) smooth brome growing (away from river deposited tailings) in agricultural soil were mixed in 1:1, 1:2, 1:4, and 1:8 dilutions (root:soil by volume) with sterilized greenhouse soil. Control treatments consisted of sterilized greenhouse soil material with no root inoculum. In the greenhouse, sudangrass, acting as a plant host, was seeded into these mixtures and its roots were examined for quantitative mycorrhizal infection to assess the impact that tailings material plays on mycorrhizal infectivity in the field.

In the 1:1 and 1:8 treatments there was significantly lower infectivity between both tufted hairgrass VAM and redtop bentgrass VAM and VAM from smooth brome. In the 1:2 there were significant differences between all three grass species' VAM. In the 1:4 treatment experimental error was probably the cause of unexpectedly high infection rates of tufted hairgrass. Similar to the 1:1 and 1:8 treatments, in the 1:4 treatment there was a significant difference between the infectivity of VAM from redtop bentgrass and smooth brome. Redtop bentgrass VAM was significantly less infective than smooth brome VAM at all inoculum levels. In all four treatments there were significant differences between smooth brome VAM and the VAM from one or both of the other two species. It was concluded that there are significant differences in VAM infectivity based on the source of VAM inoculum material. Increased examination frequency for infection and a longer growing period may yield more consistent results in similar infectivity studies.

## INTRODUCTION

Vesicular-arbuscular mycorrhiza (VAM) are a mutualistic symbiotic fungi that commonly infect species from most plant families. When nutrients or water are in limited supply VAM provide the plant with improved availability and in return receive photosynthate. Mycorrhiza are found in all geographic regions and under most soil, topographic, ecologic, (human) use, and climatic conditions.

The role and effectiveness of mycorrhiza varies with environmental conditions. Their symbiotic relation with a plant host is most successful when the plant is under some nutritive or competitive stress. Under some conditions mycorrhizal infection may be detrimental to the plant host. Deleterious conditions, such as chemical toxicity, that directly effect plant health may also directly effect mycorrhizal health.

Through inoculation of seed, plants, and soil, mycorrhizal symbiosis may be utilized to improve plant health and competitiveness in agriculture, range sciences, and land reclamation. Mycorrhiza are currently being used to enhance crop production in some areas. Current mycorrhizal "technology" for land rehabilitation is minimal, though much is known about the effects of land disturbances on mycorrhizal infection (eg. Allen and Allen 1980, Killham and Firestone 1983).

The inclusion of vesicular-arbuscular mycorrhiza in land reclamation project designs has the potential to increase revegetation success because many drastic land disturbances, such as surface mining, damage the biotic potential of the soil, including mycorrhiza. Biotic potential may be reduced by many means including the lack of topsoil, topsoil storage, soil manipulation bringing toxic materials up to the root zone, and the addition of toxic mining/milling wastes to the soil profile. As VAM form mutualistic symbiotic relationships with their plant hosts, their reduction often has measurable negative impacts on the host plant's vigor and competitiveness. Reclamation project success, therefore, may be limited or hindered where VAM relationships are reduced.

The purpose of this study was to demonstrate the effects that mine tailings derived alluvium have on VAM infection. The general design was to compare the infectivity of mycorrhiza from three grass species that had been growing in, near, and away from tailings depositions.

Vesicular-arbuscular mycorrhizal roots from tufted hairgrass, redtop bentgrass, and smooth brome growing in or near areas of alluvial tailings deposits were mixed in 1:1, 1:2, 1:4, and 1:8 dilutions (root:soil, v/v) with sterilized soil. Sudangrass, acting as a plant host, was seeded into these mixtures and its roots were examined for quantitative mycorrhizal infection to assess the impact that tailings material plays on mycorrhizal infectivity in the field.

## LITERATURE REVIEW

### Vesicular-Arbuscular Mycorrhiza

Vesicular-arbuscular mycorrhiza, like all mycorrhiza, form mutualistic symbiotic relations with their plant hosts. They commonly form endomycorrhizal relations in most plant families of the angiosperms, gymnosperms and pteridophytes. The angiosperms represent the plant group most commonly infected by VAM, with only the Chenopodiaceae, Cruciferae, Cyperaceae, and Rosedaceae rarely infected. Vesicular-arbuscular mycorrhiza form aseptate fungal structures within the root cortex of the host plant. There is no specific relation known between VAM species and plant species. Taxonomically dissimilar fungi may have similar effects on seedling growth (Johnson 1977). Mycorrhiza are common to all geographic regions of the globe and are found in most ecological systems.

Taxonomically, VAM are placed in the Endogonaceae in four genera: *Acaulospora*, *Gigaspora*, *Glomus*, and *Sclerocystis*. All are spore forming. *Acaulospora* and *Gigaspora* reproduce by chlamydospores and *Glomus* and *Sclerocystis* species reproduce by azygospores. Although they sometimes form ectomycorrhiza and are thought to be related to the VAM, the mycorrhizal forming status of the *Complexipes*, *Endogone*, *Entrophospora*, *Glaziella*, and *Modicella* genera is unknown (Hall, 1984).

Vesicular-arbuscular mycorrhizal infection is not thought to

produce any cytological or anatomical changes to the host plants' root. Occasionally, such as in onions, the infected root may be colored yellow. Mycorrhizal infection may also result in slightly reduced root size and a reduction in the number of root hairs. This reduction is presumably because the infecting fungi is taking over some of the physical gathering/absorbing capacities of the plant root. Infection does not occur in the central vascular cylinder or meristematic regions of the root and continuous infection throughout the root system rarely occurs. Infection is usually sporadic and localized (Daft and Nicolson 1969).

Vesicular-arbuscular mycorrhiza fungal structures are phycomycetous and include intercellular and intracellular hypha, vesicles, arbuscules, and reproductive chlamydospores or zygospores. These fungi form extramatrical (outside of the host root) hyphal structures but they are differentiated from those of ectomycorrhiza by the lack of defined hyphal mantle. Intercellular hypha are found in the cortical parenchyma. They spread by dilating intercellular spaces and form bundles and connections within and between individual hyphal strands. Points of hyphal passage into the root are often marked by an appressorium (extramatrical hyphal spreading) or intracellular fungal constriction. When passing between cells, VAM maintain cell wall continuity by fusing with, and invaginating into, the host cell plasmalemma. Intracellular infection is characterized by hyphae passing straight through or looping within a cell. Intracellular hyphae do not branch as a feature of hyphal extension.

Vesicles are storage organs forming terminally on hyphae both

intercellularly and intracellularly. Their cell walls are thin at first but with age the walls thicken and their cytoplasm becomes filled with lipids (fatty acids). Arbuscules form by dichotomous hyphal branching within a host cell and are sites of nutrient exchange between mycorrhiza and plant. Arbuscules survive for only a few days and then are dissolved and absorbed by the plant (lysis). The reproductive spores of individual mycorrhiza are the primary identifying feature used for species description and classification.

### **VAM Plant Relations**

A great deal of current mycorrhizal investigation centers on the basic physiology of the mycorrhiza-host plant relationship. While the mycorrhiza provides the host with improved water or mineral nutrition, the host provides photosynthate (carbohydrate) to the mycorrhiza. The mycorrhiza quickly converts these carbon compounds into fungal carbon compounds. This conversion is rapid so that there will be a constant concentration gradient from host to heterotroph (Gianinazzi-Pearson and Gianinazzi 1983). Concentration gradient and photosynthate supply going to the mycorrhiza may play a role in McGonigle and Fitter's (1988) conclusion that mycorrhiza do not always supply their host plant with increased nutrients (particularly phosphorous) and therefore are not always mutualistically symbiotic. Their experiment involved transplant of infected plants from greenhouse pots into the field. Mycorrhiza of the colonized plants may have therefore been placed into a situation where they were not biologically needed. It should be noted that the

role of mycorrhiza is not always beneficial.

Phosphate absorption is believed to be the most important mycorrhizal function. Carling and Brown (1982) indicated that increased phosphorous-absorbing surface from hyphae extending into the rhizosphere plays a role in increased phosphorous uptake. Mycorrhiza are not attributed with the ability to mobilize phosphate sources unavailable to non-mycorrhizal roots but rather they are thought to possess a pathway with a much higher affinity for phosphate than non-mycorrhizal roots (Gianinazzi-Pearson and Gianinazzi 1983). The increase in root absorbing surface provided by extramatrical mycorrhiza would also play a role in phosphorous gathering. This would be especially important when soil is dry and phosphorous is less mobile. Top-dressing with phosphorous fertilizers has been demonstrated to decrease mycorrhizal infection but the effect declined two years after application on a Pennine (England) grassland (Sparling and Tinker 1978). Graw (1979) indicated that the phosphorous compound applied, in conjunction with pH, played a role in mycorrhizal infection. Sparling and Tinker (1978) further noted that lime, nitrogen, and potassium fertilization did not appreciably affect amounts of mycorrhizal infection.

Daft and Nicolson (1969) found mycorrhizal infection under experimental conditions as high as 90% or as low as 25% (on agricultural tomatoes) with different phosphate sources and concentrations. Hayman and Mosse (1971) similarly indicated the effects of mycorrhiza and the addition of available phosphate to be closely related and somewhat similar. They found that in soils deficient in available phosphate, responses to added phosphate were similar, equal, or less than those to

mycorrhiza. The change generally depended on the soil type and texture (whether the soil was a "phosphate-fixing" soil, thus rendering the phosphorous unavailable). Soil pH influences the solubility of diverse phosphorous compounds differently and would also play a role in plant availability (as well as give contradictory results on the effects of soil pH on VAM) (Graw 1979).

There are no known exclusive VAM-host plant relationships but it has been suggested that mycorrhiza generally do not grow in the absence of a host plant (Hetrick 1984). Powell (1976), however, demonstrated that spores do produce fan-like septate hyphae without association with a host. These fan-like structures, however must undergo cytological changes before they infect a plant. These changes must occur before root penetration is possible even if the fungi is actually touching the root (Powell 1976). Vesicular-arbuscular mycorrhiza can form infections from root fragments (mycelium), spores, and sporocarps (Read et al. 1976; Sanders and Sheikh 1983) but infection rates are faster when root material is used as inoculum (Hall 1976). Johnson (1977) further demonstrated that detached hyphae were even more infective than root segments. Spores, however, are often better able to maintain their viability during long periods of storage.

Hall (1976) found that cessation of growth by a VAM dependant shrub (*Coprosma robusta*) seedling can be relieved by the application of phosphorous containing fertilizers or by VAM inoculation. When phosphorous containing fertilizers were added plants had longer growth periods compared to VAM application but plant weight was lower than when growth depended only on mycorrhiza. In terms of land reclamation



project cost and plant survival, under many conditions, mycorrhizal infection may be of greater value than fertilization in shrub and grass establishment. Because inoculum can be introduced during seeding or planting and introduction is a one time treatment, costs could be reduced and reclamation bond (release) time started earlier.

### **Inoculum and Infection**

Increasing the amount of inoculum increases the amount of root infection (Johnson, 1977; Carling et al. 1979). Carling and his co-workers reported that infection density in soybean seedlings increased as inoculum density increased until a plateau was reached: forty grams of inoculum produced the same amount of infection as twenty grams. After this plateau was reached, no additional increase was reported. They further concluded that even extremely low inoculum levels (3 spores) would, by seasons end, be able to produce root colonization and growth response equal to that produced by high inoculum (250 spores). Daft and Nicolson (1969) demonstrated that increased inoculum rates resulted in increased infection in tomato plants for a period of time--with the same peak colonization rates regardless of the initial inoculum rate. The pattern of plant growth they discovered was unique. When the tomato plant was inoculated with a high number of spores more apical leaves developed and more lower leaves were retained. Low spore inoculum plants had apical leaf development similar to uninoculated plants but more basal leaves were retained. Daft and Nicolson postulated that the mycorrhiza somehow stimulated the ontogeny and

delayed senescence of leaves. Their alternative explanation was that the effects of inoculum rate were related to nutrient uptake. As expected, phosphorous levels in the infected plants were higher than in the control plants. An important difference between the research of Carling and his co-workers and Daft and Nicolson's study is that the former measured infection in seedlings, before growth response would be detectable, and the latter examined mature plants. Carling and his associates concluded that an increase in the maximum growth yield is more probable if the plant was infected from the seedling stage. Early infection, it may be expected, would increase plant competitiveness but since infection rates plateaued within one growing season (Daft and Nicolson 1969; Johnson 1977; Carling et al. 1979) the competitive advantage was short lived.

Infection is usually associated with non-primary root development (Brown 1956; Rich and Bird 1974; Carling et al. 1979) suggesting that a fixed period of time is required for mycorrhizal penetration, elongation, and organ development. There could also be a required delay between root penetration and arbuscule development, which would indicate nutrient exchange (Sutton 1973). Sutton reported that greater than 90% of mycorrhizal infection of crop plants occurred in secondary or later roots. The potential advantages of high inoculum application and subsequent higher early infection rates include increased plant vigor and competitiveness, earlier mycorrhizal spore production (reproduction), plant growth stimulation, and in the case of annual or short lived plants (whether because of ecology or plant physiology), maximum mycorrhizal benefit within the plant's growing season (Hetrick

1984).

The weight of VAM-infected roots was determined to be greatest during summer but the highest percent infection was found during winter, suggesting there is a considerable amount of root turnover and some self-regulation mechanism in inoculum rate (Sparling and Tinker 1978). The amount (limit) of infection of most plant species is also thought to be constant (Read et al. 1976; Sparling and Tinker 1978). Powell (1976) demonstrated that spores generally germinated within 16 days given adequate conditions but sporal hyphae did not infect onion roots for long periods of time or until they were in physical contact. In contrast, hyphae from infected root segments immediately infected the onion roots. He concluded this difference between resting spores and mycorrhizal root segments is probably because of differing nutrient supplies as well as necessary cytological changes in early spore-produced hyphae (which are septate and characteristically different from root-associated hyphae). These findings are supported by Allen and Allen (1980) who indicated that infection percentages were not highly correlated with spore counts. Sites disturbed by mining often had high spore counts, even after soil mixing, but had low infection rates relative to undisturbed range. This lack of correlation suggests that many factors determine inoculum potential. These include plant species genotype, soil features (nutrients, texture, moisture), and soil microbial activity (Daft and Nicolson 1969; Allen and Allen 1980).

Of particular relevance to the current study is work by Johnson (1977) which evaluated the effects of a variety of inoculum and soil mixtures on mycorrhizal infectivity. Johnson found that the properties

of endophytes differ when growing in different soils (in older communities). Host plant growth was enhanced when plants were infected with fungi native to the tested soil. *Glomus microcarpus* (a mycorrhizal species) an effective colonizer in a pH 5.7 soil was compared with *Gigaspora gigantea* (another mycorrhizal species), which was an effective colonizer in pH 3.7 soil. Comparing infection and plant response in various soils both species of mycorrhiza produced significantly larger plants in the soil from which they were obtained (adapted). Two other experiments by Johnson, however, using different mycorrhiza and soils showed no significant difference between infection rates. These mixed results suggest that some mycorrhiza may develop greater specificities than others or soil characteristics to which mycorrhiza are specific may be altered by experimental conditions (Johnson 1977).

Similarly, Lambert and Cole (1980) concluded that there were differences between mycorrhizal species infecting white clover. Plant growth under low pH conditions was significantly greater when plants were inoculated with fungal species from acid sites (pH < 4.0). Growth of plants infected with non-acid-adapted mycorrhiza was significantly greater at higher pH. While examining the effect of pH on two plant species infected with the same mycorrhizal strain (*Glomus macrocarpus*) Graw (1979) found that plant species has a strong influence on the efficiency of VAM at different pH values and with different P-compounds. In most cases the fungus was more efficient at higher pH values. Higher pH values are generally more desirable for land reclamation but these results suggest that where lower pH is a factor, specific plant, fertilizer, and fungal combinations may increase revegetation success.

Lambert and Cole concluded that establishing mycorrhiza in mining wastes is useful if indigenous inoculum is absent or non-adapted, available phosphorous is low, and the reclamation plant species are mycorrhizal.

### Water Relations

The removal of extraradical hyphae from clover and leek was shown to decrease plant water pressure (Hardie 1985). The author concluded that the reduction in extraradical hyphae reduced plant absorptive capacity and hence plants had difficulty maintaining transpiration levels after transplanting. The experimental design was different from most other VAM response comparison studies because VAM infected plants were used for both the experimental and control treatments. After desired initial growth was achieved the extraradical hyphae of the experimental plants were removed. There is a possibility that the physical manipulation and exposure of the roots played a role in the study results but the method provides a more natural standard for VAM comparison. The advantage of using identically infected plants is that the nutritional status, particularly the phosphorous status of both plant groups is ensured (Hardie 1985). Bradley et al. (1982) and Gildon and Tinker (1983a) pointed out the need to consider the amount of available phosphorous in the soil when judging mycorrhiza's effects on plants.

Allen and Boosalis (1983) reported the presence of mycorrhiza did not affect plant dry weight but increased stomatal conductances. Stomatal closure occurred at lower leaf water potentials and after

greater desiccation in mycorrhizal plants than in non-mycorrhizal plants. *Glomus fasciculatus* increased winter wheat drought tolerance but *G. mosseae* did not, suggesting that both plant species and mycorrhiza species must be taken into consideration.

Hetrick et al (1987) reported that big bluestem benefited by mycorrhizal association under cyclic and severe drought stress but corn and sudangrass did not. They pointed out a relationship between non-amended and phosphorous amended plants; drought stressed mycorrhizal plants without phosphorous amendments were not larger than non-mycorrhizal plants that were fertilized. The plants apparently were more dependant on the mycorrhiza for phosphorous than for water relations. Nelson and Safir (1982) demonstrated that drought tolerance was improved by mycorrhizal association in fertilized onions but this mycorrhizal growth response was eliminated under drought stress in a similar experiment using corn (Hetrick et.al. 1987). Factors cited as leading to these contradictory results include the severity of drought stress, difficulty in maintaining constant laboratory conditions, soil type, and individual plant responses (Hetrick et.al. 1987).

With these factors in mind, the conditions (mycorrhizal infection, soil type, moisture regime, etc.) responsible for plant vigor or growth may be related to, but not dependant on, mycorrhizal infection. The most consistent plant responses to VAM infection occur as a result of an improved phosphorous nutrient regime (Cooper, 1984) but plant specific response to VAM infection would play an equal or greater role than soil fertility (Azcon and Ocampo, 1981).

### VAM and Reclamation

The degradation of the biotic environment as a result of land disturbances, including mining, may include reduced topsoil volume, mixing topsoil with buried and/or toxic materials, and the addition of toxic materials to the root zone. Plant succession following land disturbance also plays a role in mycorrhizal relationships. On disturbed rangeland in northern Colorado, Reeves and co-workers (1979) demonstrated that primary succession is dominated by non-mycorrhizal plants of the Chenopodiaceae and Brassicaceae families. Miller (1979) also showed that species of the Chenopodiaceae family were the dominant primary colonizers of disturbed rangeland in Wyoming. Because only one percent of the primary colonizers on disturbed lands were mycorrhizal compared to 99% on adjacent undisturbed rangeland Reeves and his associates concluded that in the disturbed (mycorrhiza deficient) rangeland, non-mycorrhizal species were more competitive than the mycorrhizal species. Furthermore, the non-mycorrhizal species may hinder successional stages because they do not provide an inoculum source for later successional species which require mycorrhizal associations for survival (Moorman and Reeves 1979; Reeves et al. 1979).

Miller (1979) examined VAM infection of plants on disturbed and undisturbed/sites in the red desert (Wyoming) and concluded that reproductive strategy and mycorrhizal infection play major roles in species establishment and competitiveness following disturbance. Of note was halogeton (*Halogeton glomeratus*) which comprised over 90% of total plant cover at the site. VA mycorrhizal spores (*Glomus* spp.) were

present but through an unknown mechanism, possibly allelopathy by halogeton, the spores were not inoculating plants at the site. A plant's ability to become colonized with mycorrhiza would be of no benefit in an environment such as this. Therefore, a plant must assume a non-mycorrhizal ruderal reproductive strategy to establish (Miller 1979). In their study of Colorado rangeland, Reeves et al. (1979) found that the Poaceae family is one of the dominant families in primary succession. While most of the plants of Poaceae are mycorrhizal, only a limited number are commonly found growing in toxic environments.

One explanation for non-mycorrhizal plant species and plant communities was presented by Tester and co-workers (1987) who proposed that fungitoxic compounds in cortical root tissue or root exudates may reduce plant susceptibility to fungal infection. They additionally proposed that infection may be limited or halted by a lack of root exudation (the VAM not receiving adequate nutrients to develop or sustain infection), poor exudate quality, or the lack of compounds required in minute (putative) quantities to "signal" infection. Tester and his associates' final theory suggests that cell wall or lamellar interaction prevents mycorrhizal penetration. This hypothesis is supported by their observation of roots heavily encircled by hyphae but with no infection into the roots.

Reducing topsoil through removal or mixing reduces the number of spores and the amount of available inoculum material. Topsoil storage primarily reduces the viability of root inoculum material but also, to a lesser degree, spore vigor (Rives et al. 1980; Liberta 1981; Hardie 1985). Allen and Allen (1980) studied the natural reestablishment of



VAM following stripmine reclamation. They found that mixing topsoils with subsoils reduced initial spore counts by 5% to 25% (compared to undisturbed sites) but that mycorrhizal infection was up to 50% of undisturbed after three years. Waaland and Allen (1987), however, suggested that the kind of soil substrate was more important than time in determining conditions for spore inoculum. Comparing 1 to 6 year and 10 to 31 year old reclaimed soil, reclaimed spoil, and undisturbed range they found that there was no relationship between time from disturbance/reclamation and spore density. The most important of the specific experiment results was that topsoiled sites almost always had significantly higher spore counts than reclaimed or orphaned sites indicating the importance of topsoil salvage and creating a favorable microbiotic environment through reclamation.

### VAM and Soil Condition

Vesicular-arbuscular mycorrhizal response to toxic materials are the most difficult mycorrhiza-reclamation relationship to study because many factors play a role in plant response. These factors include plant species, mineral nutrition, soil type and chemistry, water supply, and the type and degree of toxin. Tufted hairgrass (*Deschampsia caespitosa*), the plant species being considered in this investigation, is known for its metal tolerance (Cox and Hutchinson 1980; Cahoon 1983).

In their experiment examining weedy and colonizer species' mycorrhizal infection, Pendleton and Smith (1983) discovered that over one-half of the 75 weedy species they evaluated were mycorrhizal. The

presence or absence of infection was strictly within family taxonomic divisions and they concluded that family connection was more important than weedy habit in determining mycorrhizal infectivity. Flat semiarid sites were dominated by non-mycorrhizal species whereas rocky sloped sites were dominated by mycorrhizal species having deeply penetrating root systems. These results, particularly with respect to the non-mycorrhizal semi-arid sites, indicate the importance of the role that water availability plays in determining the percent colonization and total number of mycorrhizal colonizers (Reeves et al. 1979; Pendleton and Smith 1983; Hetrick 1984).

Among the past theories to why tufted hairgrass and colonial bentgrass (*Agrostis tenuis*), another metal tolerant species, are often found growing in metal toxic and low pH areas is that mycorrhiza act as filters, protecting the plant from the metals (Gildon and Tinker 1981; Bradley et al. 1982; Gildon and Tinker 1983a; Killham and Firestone 1983; Ferns 1984). The general conclusion of these studies of the mycorrhiza-as-filters theory, however, is that VAM do not protect the plant from heavy metal contamination and may in fact act as a pathway for increased plant uptake. Studies examining the role of mycorrhiza in toxic environments can be broken into two groups: 1) mycorrhiza-plant symbionts that have evolved in a toxic environment, and 2) mycorrhiza-plant symbionts that have survived together in an environment rapidly made toxic by human activities such as mining/milling.

In serpentine soils, which are characteristically high in iron, nickel, and magnesium but low in nitrogen, potassium and phosphorous, Hopkins (1987) determined that 26 of 27 established herbaceous species

were mycorrhizal (23 of these mycorrhizal species had almost solid infection of the cortex). Annuals made up 84.4% of the herbaceous cover. She proposed that shallow soil (20cm) and poor parent material (ultra-mafic rock) may have contributed to nutrient and water stress thus creating an obvious niche for mycorrhiza. The uniqueness of heavy colonization of annuals in poor soil also suggests that the role of plant density, early colonization, and rapid colonization were factors allowing the symbiosis to succeed (Hopkins 1987).

Gildon and Tinker (1981,1983a,b) and Bradley et al. (1982) also performed experiments on naturally occurring toxic soils. Bradley and co-workers concluded that the hyphal complexes of the mycorrhiza provided adsorptive surfaces for metals, thereby protecting the plant by effectively binding toxic metals in the root cortex. In two of their experiments, Gildon and Tinker (1981, 1983a) concluded that a particular strain of clover-infecting mycorrhiza that had grown naturally on heavily zinc and cadmium contaminated sites was protecting its host from absorbing some of the metals. The second of the two papers (Gildon and Tinker 1983a), however, suggested that in the act of filtering metals the mycorrhiza may not be providing adequate phosphorous to the plant. Less promising were the conclusions of Gildon and Tinker's third paper (1983b) which indicated that copper uptake in leeks could be reduced by decreasing mycorrhizal infection rates by adding phosphorous.

In contradiction to the above literature, studies of mycorrhiza-plant relations in environments made toxic by man suggest that mycorrhiza may increase plant absorbance of heavy metals. Plants infected with VAM and growing in soil contaminated with acidic and heavy

metal depositions were shown to have higher tissue metal levels and significantly reduced growth compared to non-mycorrhizal controls (Killham and Firestone 1983). These authors found that under the conditions of a pH of 5.6 and high metals, mycorrhizal and non-mycorrhizal plants had similar metal loads (especially copper and nickel, and to a lesser degree, lead and zinc). The same metal load in plants grown in materials exhibiting pH values less than 3.0 resulted in 257% and 309% increases in copper and nickel concentration in mycorrhizal plants. This is supported by Ferns (1984) who found that VAM from plants growing in a tailings pond increased transportation of toxic metals to a non-metal or low pH tolerant host.

Killham and Firestone (1983) noted that metals entering the soil from the atmosphere could be complexed in a high clay or organic soil but would be more available in sandy soils. Where atmospheric or uncomplexed metals are common, such as around smokestacks and milling operations, therefore, mycorrhiza would not be a desirable land reclamation feature.

In further support of these explanations of metal tolerances, Cahoon (1983) examined the intraspecific differences in heavy metal accumulation and distribution in tufted hairgrass. He concluded that a race of tufted hairgrass that was collected on a metalliferous waste site had greater metal tolerance (accumulated less cadmium or copper) than a race grown from commercial seed. Physiological differences in the uptake and transport of metals within the plants were also noted.

The general trend in the literature is that VAM infection and spore production increase, to a point, as soil pH increases (Graw 1979).

Change in pH is likely to affect plant available phosphorous, thereby influencing mycorrhizal colonization. Mosse and Phillips (1971) demonstrated that high levels of phosphorous or nitrogen may inhibit VAM infection rates. A plant's condition, with regard to nutrients and water, would determine its degree of mycorrhizal infection. It has therefore been concluded by a number of authors that the nutritional health of the plant directly controls infection rate rather than the fertility of the soil (Menge et al. 1978; Hetrick 1984).

## METHODS AND MATERIALS

### Study Design

A greenhouse study was implemented to determine whether the inoculum potential of VAM is impacted when the fungus is found on plants growing in an inhibitory tailings environment. The general strategy of the design was to mix VAM-colonized roots from plants growing in and near areas of tailings deposition (as inoculum material) with sterilized soil and grow host plants whose roots would be examined for infection. Roots were chosen as the inoculum material because much of the soil associated with soil/spore inoculum for tufted hairgrass could contain tailings material. Additionally, because of time constraints and to minimize site disturbance spore inoculum was not used.

Plant roots were collected from 1) tufted hairgrass (*Deschampsia caespitosa* (L.) Beauv.) growing in a mixture of soil and tailings materials deposited by the Clark Fork River, 2) Redtop bentgrass (*Agrostis alba* L.) growing near the river but not directly in tailings contaminated soil, and 3) Smooth Brome (*Bromus inermis* Leyss.) growing in agricultural soil that has been unaffected by tailings carried in the river. Root collection depth ranged from 15 to 60 centimeters. Soil was determined to be tailings enriched based on Soil Conservation Service soil descriptions and metal contamination data from Rice and Ray

(1984), and soil pH data taken from across each collection area. Because *Agrostis alba* tends to take on the aspect of *A. stolonifera* and the two species appear to intergrade (Hitchcock and Chase 1971), *Agrostis alba* will refer to both species for the purposes of this paper. Root material from each grass species was mixed with sterilized soil in dilutions of (roots:soil, by volume) 1:1, 1:2, 1:4, and 1:8 and seeded with sudangrass (*Sorghum sudanense* (Piper) Stapf.). Roots from each of the plants and treatments were harvested and examined for quantitative VAM infection at two different growth stages (two leaf and five leaf).

### Site Description

*When I first reached Montana, the Deer Lodge Valley was one of the most beautiful stretches of bunch grass country imaginable. The grass waved like a huge field of grain.*

Conrad Kohrs (NPS 1987)

John Grant brought a herd of cattle to the Deer Lodge Valley in the early 1800's. These cattle, along with others acquired on immigrant trails such as the Mormon and Oregon Trails, formed the foundation for Montana's Cattle industry. Around the turn of the century, the Ranch had holdings of 30,000 acres and grazed one to five million acres throughout the Northern Rockies states. The Grant-Kohrs Ranch National Historic site was established to provide an understanding of the frontier cattle era and preserve the Grant-Kohrs ranch because of its historic significance (NPS 1987). The Ranch is located in Deer Lodge, Montana (Powell County T8N, R9W, Sec28) (Figure 1).

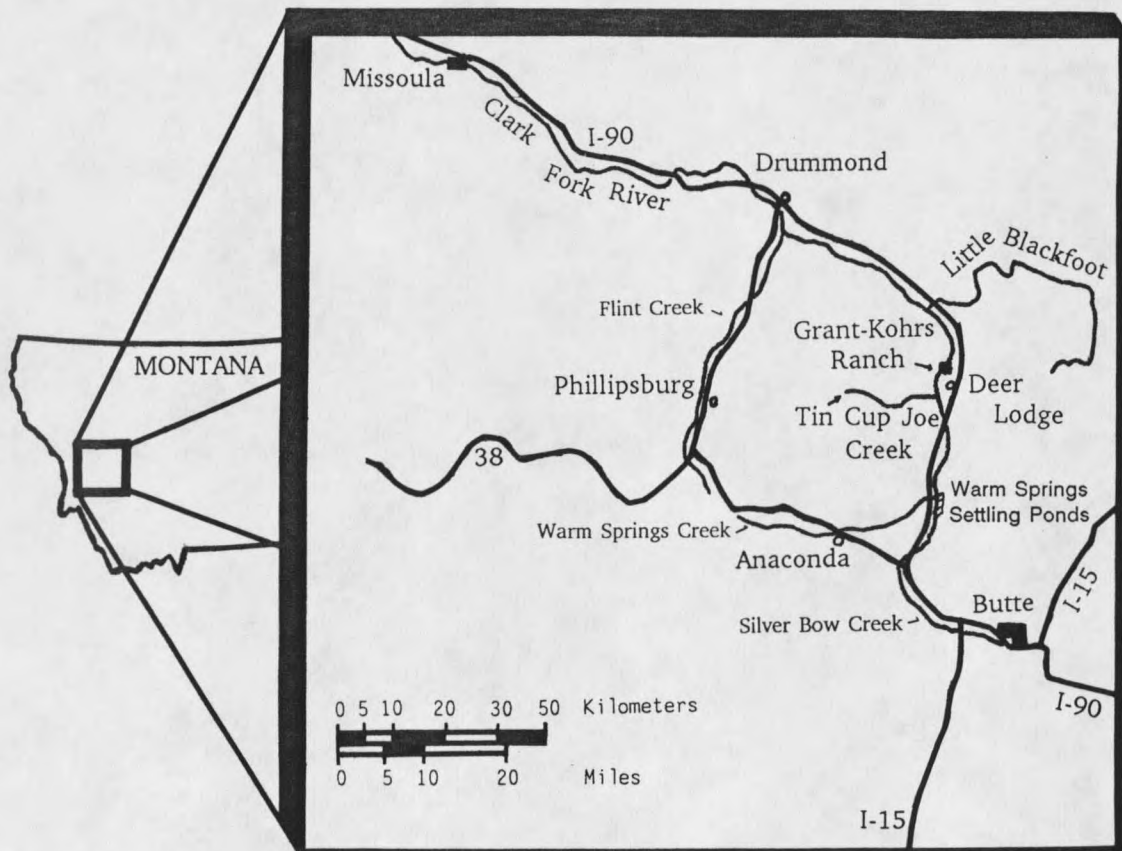


Figure 1. Location of the Grant-Kohrs Ranch National Historic site (study site).

Samples were collected along the Clark Fork River in the west central part of the Grant-Kohrs Ranch (Figure 2). The headwaters of the Clark Fork is formed by the convergence of Warm Springs Creek (SW from Anaconda), Silver Bow Creek (SE from Butte), and smaller creeks and streams. The Clark Fork forms the drainage of the west slope of the continental divide and the east slope of the Flint Creek Range (Pintler Mountains). The Silver Bow and Warm Spring Creeks drain the mining and smelting areas of Butte and Anaconda and together with the Clark Fork, have a history of carrying and spreading mining waste and tailings materials along their floodplains.



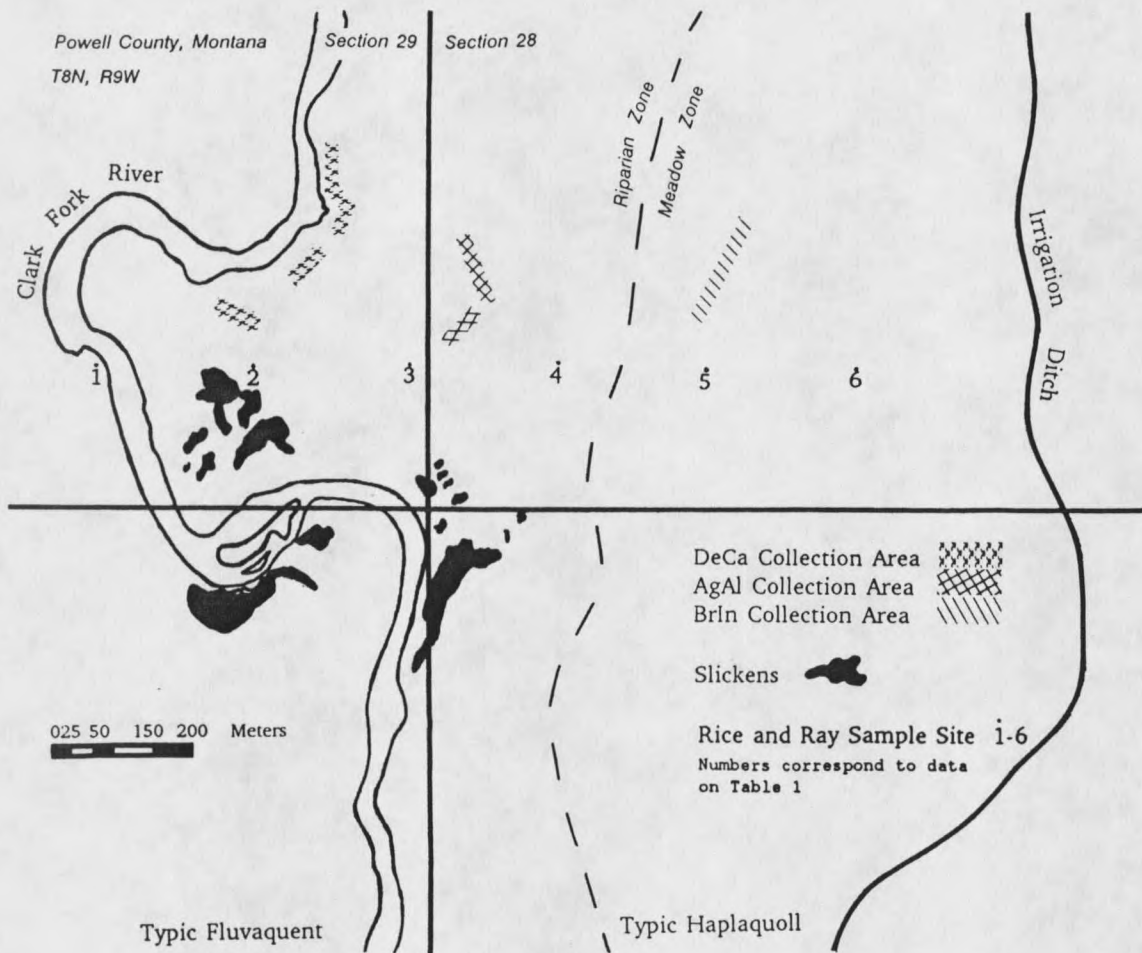


Figure 2. Sample collection locations at the Grant-Kohrs Ranch.

### Soils

The soils of the west central part of the ranch, where tufted hairgrass and redtop samples were taken, were classified as "Typic Fluvaquent, mixed; frigid; undifferentiated" (SCS 1988). The cultivated soil where smooth brome roots were taken was classified as Typic Haplaquoll, fine-loamy, mixed. A number of areas were classified as "slickens" by the Soil Conservation Service. These were defined as "an

undifferentiated soil type consisting of accumulation of fine-textured materials, such as are separated by placer mine and ore mill operations. Slickens from ore mills consist largely of freshly ground rock that commonly has undergone chemical treatment during milling or smelting processes" (SCS 1988). Slickens sites determined by Rice and Ray (1984) are identified in Figure 2. Rice and Ray discovered that arsenic levels in sediments in the slickens and grasses (tufted hairgrass) growing near their perimeter were as high as originating adjacent to actual smelter sites.

Preparation for soil pH determination consisted of mixing 50 grams of soil (dry) with 100 milliliters of distilled water and letting the mixture stand for 45 minutes (Ruddell and Montagne). Five samples were collected from across each of the three plant collection areas. Hydrogen ion concentrations at the collection sites ranged from 4.5 to 6.0 for tufted hairgrass (average 5.0), 5.0 to 6.5 for redtop bentgrass (average 5.5), and 5.0 to 6.8 for smooth brome (average 6.4). These numbers indicate little change from those values determined by Rice and Ray (1984) and Ray (1985). Ray reported pH values to range from approximately 4.2 to 6.8.

There was no change in floral composition, soil description, or pH between the 1984 survey and those established in the course of this study (Figure 2). For the descriptive purposes of this study the heavy metal concentrations they reported were accepted as accurate. Total soil and plant accumulated copper, arsenic, and cadmium concentrations for Rice and Ray (1984) sample sites located close to the root collection zones for this study are presented in Table 1. Table 1 also

presents metal concentrations they found at the Tin Cup Joe Creek Check Plot (Figure 1). Rice and Ray established this check plot on the fluvial contour of the Tin Cup Joe Creek because the area had no history of water borne metal contamination and was formed from similiar parent material (Quartenary glacial outwash plain) as soils of the Grant-Kohrs Ranch. Metal contamination levels were similiar (highest metals closest to the river, decreasing moving away from the river) along the length of the Clark Fork that they examined.

There was a metal gradient in soils decreasing away from the Clark Fork River. This was consistent with floral composition (Rice and Ray 1984). Tufted hairgrass was found most commonly along the Clark Fork in inhibitory zones of flood event tailings deposition. Redtop bentgrass was found in transitions between the riparian and meadow zones. It was also found in isolated clusters along the river.

Rice and Ray (1984) reported that the concentrations of copper, cadmium, and arsenic in soils along the Clark Fork River at the Grant-Kohrs Ranch were greatly elevated relative to their control plots. They demonstrated that metal laden sediments were carried by the Clark Fork and deposited on the floodplain of the Grant-Kohrs Ranch. The highest metal concentrations were found in the riparian floodplain, particularly along the river and in old channels and sloughs where sedimentation had taken place. The highest metal concentrations in the meadows were found adjacent to the irrigation ditch and were concluded by Rice and Ray (1984) to be a result of flood overflow and sediment deposition or sediment maintenance removal. These concentrations could also be a result of irrigation.











































































