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Dissertation

Relationships Among Nitrogen Availability, Vesicular-Arbuscular Mycorrhizae, and *Bromus tectorum* in Disturbed Rangeland Sites in Colorado

Submitted by

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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Fall, 2002

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WE HERBY RECOMED THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY **ABDULAZIZ A. AL-QARAWI** ENTITLED **RELATIONSHIPS AMONG NITROGEN AVAILABILITY, VESICULAR-ARBUSCULAR MYCORRHIZAE, AND *BROMUS TECTORUM* IN DISTURBED RANGELAND SITES IN COLORADO** BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF **DOCTOR OF PHILOSOPHY**.

Committee on Graduate Work

Adviser

Department Head

Abstract of Dissertation

Relationships Among Nitrogen Availability, Vesicular-Arbuscular Mycorrhizae, and *Bromus tectorum* in Disturbed Rangeland Sites in Colorado

This dissertation has three main parts. The first was a study of effects of nitrogen availability and the exotic weed *Bromus tectorum* L. on the density of vesicular-arbuscular mycorrhizae (VAM) fungi in disturbed land in Colorado. Field and laboratory studies were designed to measure the change in density of VAM fungi in response to available N levels and plant species present. Three nitrogen treatments were established: 1) added nitrogen (N+); 2) control (no added nitrogen, C); and 3) sucrose added to reduce nitrogen availability (N-). Two common grasses, the exotic annual weed *B. tectorum* and a native perennial *Agropyron smithii* Rydb., were examined in this study. Soil samples beneath each species were taken from each N treatment plot. Both nitrogen availability and plant species were correlated with change in VAM fungal density. The N+ treatment was associated with decreased VAM fungal propagules in soil beneath *B. tectorum*. Similarly, there was a general trend but not a significant association between N+ treatment and a decrease in VAM fungal propagules in soil beneath *A. smithii*.

The second part was a study to determine changes in VAM fungal abundance and infectivity following disturbance. Three different methods -1) a bioassay to measure mycorrhizal inoculum potential (MIP), 2) a bioassay to measure most probable number (MPN), and 3) direct spore counts (SC) - were used to determine the VAM fungal populations in the same soil samples collected from patches dominated by *B. tectorum* and adjoining patches dominated by perennial populations of native species at two different sites (Central Plains Experimental Range and Piceance Basin). All three methods showed significantly reduced VAM fungi population beneath *B.*

tectorum. The success of *B. tectorum* appears closely tied to its ability to reduce and perhaps change the beneficial VAM fungal population indigenous to an area.

The third part was a study to determine the effects of VAM fungi on competition between *B. tectorum*, and a perennial wheatgrass (*Pseudoroegneria spicata* (Pursh.) A. Löve) and to determine the relative mycorrhizal dependency (RMD) for both species. Both species were grown in monoculture and mixed pots, with (M+) and without (M-) VAM fungal inoculum. Both species developed substantial mycorrhizal colonization when grown on M+ soils. Mycorrhizal colonization increased growth (dry shoot and root biomass) of both species. Competition between the two species resulted in elevated colonization in *B. tectorum* roots and reduced colonization in *P. spicata* roots when compared to monoculture pots. *B. tectorum* had higher RMD than *P. spicata*. They did not show significant competition between them, except the increased colonization in *B. tectorum* roots and the reduction in colonization in *P. spicata* roots during competition.

Mycorrhizal inoculum generally did not reverse the competitive outcome, but did confer varying advantages in terms of increased dry biomass to the mycorrhizal *B. tectorum*. Moreover, the response to mycorrhizal colonization and dry shoot or root biomass in monoculture and mixed pots were not correlated.

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Acknowledgments

I would like to express my sincerest thanks to the members of my graduate committee, Dr. Brent Reeves, Dr. Mark Paschke, and Dr. Wayne Leininger, and to my advisor, Dr. Edward Redente, for their invaluable help in the successful completion of my degree. Additionally, I would like to thank Dr. Reeves, Dr. Redente, and Dr. Paschke for providing an excellent research environment, encouragement, helpful suggestions, and great assistance in the preparation of this manuscript.

I dedicate this dissertation to my father who loved me and raised me to become the person I am today, to my wife, Badreyah Al- Mohaiysen, for her assistance, patience, and understanding throughout my graduate study, and to my children, Omar, Nahlah, Nehal, and Abdullah, for their support and sacrifice.

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Introduction

In order to improve ecosystem productivity and sustainability, land managers sometimes need to replace annual exotic species with perennial grass species (Roberts 1991). This may be difficult because exotic species may be better adapted to disturbed conditions than native species (Daubenmire 1970; Hull and Hansen 1974) and the factors and processes that determine the development and maintenance of a plant community are complex. Climate, nutrient status of soil, plant and animal species interactions, and the soil microbial community influence ecosystem development and stability following a disturbance. Reports on the dynamics of plant and soil interactions during old-field succession are many (Bazzaz 1979; Crowell and Boerner 1988; Vitousek et al. 1989; Koide and Li 1991b; Paschke et al. 2000). However, the dynamics of important plant-soil intermediaries such as saprobic and symbiotic microbes have received less attention. Studies of microbial dynamics during succession can provide insights into some of the important factors and processes regulating ecosystem development. Many ecological processes, including those associated with plant-microbial interactions, influence the rate and direction of succession (Reeves and Redente 1991). The formation of mycorrhizae - the symbiotic relationship between mycorrhizal fungi and roots - is an important process that appears to influence plant species composition during succession (Walker and Chapin 1987).

There are at least 7 different types of mycorrhizae (Reeves and Redente 1991). As noted by Trappe (1981), "About 95% of the world's plants species belong to families that are typically mycorrhizal - the great majority of these form Vesicular-arbuscular mycorrhizal (VA) endomycorrhizae." Vesicular-arbuscular mycorrhizal

(VAM) fungi, sometimes abbreviated AM fungi for arbuscular mycorrhizal fungi, enhance growth and development of many plant species (Gerdemann 1968; Mosse 1973), and the role of mycorrhiza in plant nutrition (Mosse 1973; Smith 1980) and water relations is very important (Nelsen and Safir 1982). VAM fungi may improve water absorption capacity (Menge et al. 1978; Nelsen and Safir 1982; Allen and Allen 1986; Allen 1991), and assist plants in uptake of nutrients (Mosse 1973; Lambert et al. 1979). Plant nutritional and isotope experiments demonstrate that mycorrhizal fungi absorb from the soil and translocate to plants N, P, K, Ca, S, Zn, Cu, and Sr (Gray and Gerdemann 1973; Sanders et al. 1975, Cooper and Tinker 1978; O'Keefe and Sylvia 1991). Mycorrhizae often improve plant growth and survival by facilitating uptake of nutrients and increasing drought tolerance (Johnson and Hummel 1985).

All terrestrial plants require an exogenous source of essential mineral nutrients - "elements" - from the soil and mycorrhizae exert their influence by altering mineral uptake in plants. Major limiting elements, "macronutrients", include nitrogen (N), phosphorus (P), and potassium (K), and are often limited in that order. Differences in nutrient availability patterns can lead to ecophysiological responses and to changes in the competitive abilities of plant species (Fitter 1982; Wedin and Tilman 1990; Grime 1994). Because soil nitrogen is a limited element for many species, its availability is a major determinant in influencing plant species composition (Tilman 1987; McLendon and Redente 1992; Redente et al. 1992; Paschke et al. 2000), plant competition (Vitousek and White 1981), and microbial community structure (Klein et al. 1996). Increasing available nitrogen encourages plant species that are adapted to take advantage of excess resource enrichment (Burke and Grime 1996; Paschke et al. 2000). In semiarid habitats plants that dominate nitrogen enriched areas are often the less desirable, exotic annual species (Lowe 2000; Paschke et al. 2000).

Although a few ruderal plant species are non-mycorrhizal, the majority of terrestrial plants form mycorrhizae and some plant species (obligate mycotrophs) appear to be more dependent on mycorrhizal fungi than other species (facultative mycotrophs) (Smith and Read 1997). Some researchers reported a general increase in plant dependency on mycorrhizae as succession progresses (Johnson et al. 1991; Panknow et al. 1991).

Several authors suggested that mycorrhizal fungi influence plant succession and community structure (Miller 1979; Reeves et al. 1979; St. John and Coleman 1983; Allen and Allen 1984; Benjamin and Allen 1987; van der Heijden et al.1998). Mycorrhizae are important in structuring plant communities (St. John and Coleman 1983) because they affect such processes as plant competition (Allen and Allen 1984), phenology (Allen and Allen 1986), and interspecific nutrient transport through hyphal links (Newman 1988). In addition, evidence suggests that above ground plant diversity is a function of the diversity of the mycorrhizal community, and that VAM fungi are important in promoting plant species diversity both in controlled experiments (Grime et al. 1987; Read 1998; van der Heijden et al. 1998) and in field studies (Newman and Reddell, 1988; van der Heijden et al. 1998). In some field studies, mycorrhizal fungal colonization of roots increases growth and survival of plants (Hayman and Mosse 1979; Call and Davies 1988). Many laboratory experiments indicate that a number of plant species benefit from vesicular-arbuscular (VA) mycorrhizal associations in terms of growth (Koucheki and Read 1976; Gange et al. 1990).

Recently Hart et al. (2001) offered two alternative hypotheses regarding the relationship between plant succession dynamics and VAM fungi. Their "Driver" hypothesis suggests that interactions within the fungal community are responsible for change in the plant community. In their alternative "Passenger" hypothesis, VAM fungal communities are the result of changes in the plant communities. In other words, it is difficult to resolve whether the dynamics of VAM fungi are the cause (Driver hypothesis) or consequence (Passenger hypothesis) of plant dynamics. They concluded that plant communities might operate at some level between these extreme hypotheses.

Goodwin (1992) and Richardson et al. (2000) discussed the potential role of mutualisms in plant invasions. They suggested that mycorrhizal fungi might facilitate invasions. Clearly, if mycorrhizae affect plant community processes, then they may play an important role in secondary succession.

Mycorrhizal fungi appear to be important in succession in rangeland systems that have been radically altered. Disturbed, semiarid lands are often invaded by either nonmycorrhizal or facultative mycorrhizal plants species such as cheatgrass (*Bromus tectorum* L.) (Wicklow-Howard 1994). VAM fungi can markedly influence the outcome of competitive interactions among plants (Allen and Allen 1986; Grime et al. 1987). The role of mycorrhizal fungi in both seedling establishment and competitive interactions among the seedlings of native and alien grasses is in need of study.

Mycorrhizal fungi provide a pathway for nutrient uptake, for nutrient transfer between plants, and may be important in stabilizing nutrient availability and in aiding nutrient cycling processes at the ecosystem level (Chiarello et al. 1982; Read 1984). Direct nutrient transfers between plants and mycorrhizal fungi may affect succession (Allen et al. 1981; Ames et al. 1983).

Reeves and Redente (1991) concluded that mycorrhizae have an essential role in controlling and directing plant community structure. The absence of VAM fungal inoculum may delay the colonization of the site by obligatory mycotrophic plant species that typically establish later in succession (Reeves et al. 1979; Miller and Jastrow 1992). Johnson et al. (1991) found that the species composition of VAM fungal communities changed during secondary succession of abandoned fields. They noted that plants characterizing late successional stages had a greater dependence on mycorrhizae in terms of growth benefit from infection while those of intermediate stages have less dependence, and mycorrhizal fungi negatively impact the non-mycorrhizal annuals of the first stages of succession. The rate of succession might be related to the increase of mycorrhizal inoculum with time. Reeves et al. (1979) and Allen and Allen (1980) suggested that recovery of disturbed ecosystems was dependent on the re-establishment of VAM fungi.

The role of VAM in phosphorus nutrition of plants is well documented (Mosse 1973; Allen 1991), but far less work has been done on the effects of nitrogen availability and VAM. In experimental manipulations of phosphorus and mycorrhizal plants, the VAM fungi are beneficial or essential for the uptake of adequate amounts of phosphorus when phosphorus is limiting. The experimental work with nitrogen and VAM fungi is less definitive, and there are few generalizations regarding the response of plants with VAM fungi to nitrogen addition. Indeed, some of the reported responses are contradictory. The amount of nitrogen available to the plant appears to be important in determining the type of response (Azcon et al. 1982; Brown et al. 1981), but the form of nitrogen also is important.

The objectives of this research were to investigate the role of nitrogen availability and the interplay of availability of nitrogen with plant community structure and mycorrhizal status in a semiarid environment. Previous researchers have shown that when nitrogen availability increases in semiarid ecosystems, the annual species (often- exotic annual weeds) will dominate, compete with, and even exclude native late-seral species (Redente et al. 1992; Paschke et al. 2000). This research was based on the following hypotheses:

H_1. **The density of VA mycorrhizal fungal communities will be reduced when nitrogen availability increases above the average level present in the soil, and when an annual exotic weed (*Bromus tectorum*) dominates and competes with native perennial species.**

H_2. **Conversely, when nitrogen conditions are low and native, late-seral species better adapted to these conditions dominates the community; these plant species will promote increased density of VA mycorrhizal fungi.**

H_3. **The three methods, Mycorrhizal Inoculum Potential (MIP), Most Probable Number (MPN), and Spore Counting (SC), used to quantify the relative VA mycorrhizal fungal of propagule densities would give similar results.**

H_4. **In interspecific competition between *B. tectorum* plants and a native perennial mycorrhizal species (*P. spicata*), the VAM fungi will colonize the roots of *P. spicata* roots more than the *B. tectorum* roots.**

H_5. **The competitive ability of *B. tectorum* would decrease and that the competitive ability of the native would increase in the presence of VAM fungi.**

H_6. ***Pseudoroegneria spicata* is physiologically capable of maximizing growth in the presence of VAM fungi, in contrast to *B. tectorum* which performs comparatively better in the absence of VAM fungi.**

Specific questions associated with these hypotheses were:

1. Does reducing nitrogen availability increase VAM fungal density?

2. Does reducing *B. tectorum* increase VAM fungal density?

3. How do the traditional methods used to quantify the relative densities of colonizing propagules of VA mycorrhizal fungi, namely, Mycorrhizal Inoculum Potential (MIP), Most Probale Number (MPN), and Spore Counting (SC), correlate and/or differ?

4. Does the exotic annual species, *B. tectorum*, depend on VAM fungi to compete with native species?

This dissertation is organized in four chapters. Following the Introduction (*Chapter 1*), several experiments were preformed to address these specific questions. The first two questions are addressed in *Chapter Two.* This chapter discusses field and growth-chamber experiments and laboratory investigations of the effects of nitrogen availability and plant type on VAM fungal density. The experiments reported in *Chapter Three* addresses question 2, and tested the effect of *B. tectorum* dominance on density of VAM fungi at two different locations in Colorado. Also, in *Chapter Three*, the different methods used to quantify the relative densities of colonizing propagules of VAM fungi are addressed. Results from bioassays to measure Mycorrhizal Inoculum Potential (MIP) and Most Probable Number (MPN), and Spore Counting (SC) of the same soil samples were used to compare methods and determine the advantages and disadvantages of each method. *Chapter Four* focuses on question four, the association of *B. tectorum* and VA mycorrhiza and competition with native species. This chapter reports on the results of experiments where *B. tectorum* was planted in monoculture and in competition with a native species, bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh.) A. Löve) in soils, with and without VAM fungi.

Chapter 2

Effect of Nitrogen Availability and Plant Species on Vesicular-Arbuscular Mycorrhizal Population

1. Introduction

Because terrestrial plants require an exogenous source of mineral nutrients, differences in nutrient availability and acquisition patterns often lead to ecophysiological responses and to changes in the competitive abilities of plant species (Fitter 1982; Wedin and Tilman 1990; Grime 1994). Soil nitrogen often is a limiting element for many species, and its availability plays a major role in influencing plant species composition (Redente et al. 1992; Tilman 1987), plant competition (Vitousek and White 1981), and microbial community structure (Klein et al. 1996). Increasing available nitrogen encourages plant species that are able to use excess resource enrichment (Huenneke et al. 1990; Milchunas and Lauenroth 1995; Burke and Grime 1996; Paschke et al. 2000). Often plants that dominate a nitrogen enrichment area are less desirable, exotic species (Lowe 2000).

Disturbance of an area alters nutrient availability. Secondary succession is the ecological process by which ecosystems recover following disturbance. Although secondary succession has long been recognized as one of the fundamental processes of ecology, and many examples have been described in the literature, the mechanisms controlling the process are still poorly understood, especially for arid and semiarid ecosystems. Plant communities often change during secondary succession in response to changes in soil nutrient availability (Tilman 1987), and these changes in soil nutrient availability during secondary succession are well documented (Lamb 1980). Lowe (2000) cited many studies that show that increasing nitrogen availability enhances the dominance of certain plant species that have the ability to respond rapidly to resource additions (Bobbink and Willems 1988; Huenneke et al. 1990; Wilson and Gerry 1995; Milchunas and Lauenroth 1995; Burke and Grime 1996).

Previous experiments at semiarid sites (McLendon and Redente 1992; Paschke et al. 2000) showed that the level of nitrogen availability greatly alters aboveground plant community structure. Nitrogen availability can be augmented with fertilization and can be reduced by adding a carbon source. The addition of carbon reduces biologically available nitrogen by stimulating microbial immobilization and microbial growth. Decreasing nitrogen availability is hypothesized to be disadvantageous to early-seral species and to provide an advantage to late-seral species (Wedin and Tilman 1990; Redente et al. 1992; Paschke et al. 2000).

Bromus tectorum L. (downy brome or cheatgrass) is an invasive species and considered a pest. *B. tectorum* responds positively to nitrogen fertilization (Rasmussen 1995). Surface applied nitrogen increases *B. tectorum* seedling survival (Rasmussen 1995), growth, and yield (Nesse and Ball 1994; Paschke et al. 2000), and increases the number of *B. tectorum* culms m^{-2} (Anderson 1990, Rasmussen 1995).

Plant communities also are influenced by mycorrhizal fungi because of variation among plant species in their response to these symbionts (Reeves et al. 1979; Anderson et al. 1984; Allen and Allen 1986; Benjamin and Allen 1987; Koide and Li 1991a; van der Heijden et al. 1998). In semiarid ecosystems, heavily disturbed soils often are invaded by either nonmycorrhizal (Miller 1979; Reeves et al. 1979) or facultative mycorrhizal alien annuals such as *B. tectorum* (Wicklow-Howard 1994). The outcome of competitive interactions among plants can be markedly influenced by vesicular-arbuscular mycorrhizal (VAM) fungi (Allen and Allen 1986; Grime et al. 1987; Bever et al. 2001). Mycorrhizal fungi appear to play an important role in succession in semiarid rangeland systems that have been radically altered. However, with a few exceptions from other ecosystems such as the tallgrass prairie (Smith et al. 1998), few definitive data that support this contention are available from field experiments.

The correlation between changes in population of mycorrhizal fungi and plant succession has been shown by several authors (Miller 1979; Reeves et al. 1979; Allen and Allen 1984; Benjamin and Allen 1987; van der Heijden et al.1998). Direct nutrient transfers between plants and mycorrhizal fungi may affect succession (Allen et al. 1981; Ames et al. 1983). Reeves and Redente (1991) concluded that mycorrhizae have an essential role in controlling and directing plant community structure. The absence of VAM fungal inoculum can delay the colonization of a site by obligatory mycotrophic plant species that characterize later succession stages (Allen 1984; Miller and Jastrow 1992).

Mycorrhizal fungi provide a pathway for nutrient uptake and transfer between plants and may be important in stabilizing nutrient availability and in aiding nutrient cycling processes at the ecosystem level (Chiarello et al. 1982; Read 1984). The role of VAM in phosphorus nutrition of plants has been known for decades (Mosse 1973; Allen 1991), but less work has been done on the effects of nitrogen availability and VAM. The influence of nitrogen on mycorrhizal associations is not clearly understood (Bååth and Spokes 1989). Sylvia and Neal (1990) suggested that plant nitrogen demand, like plant phosphorus demand, promote root colonization by VAM fungi.

VAM fungal species are sensitive indicators of nitrogen enrichment (Egerton-Warburton and Allen 2000). Field studies indicate that nitrogen fertilization decreases the root fraction colonized and the number of spores produced by VAM fungi in cultivated grass (Jensen and Jakobsen 1980). In greenhouse studies, nitrogen has been reported to increase (Azcon et al. 1982; Johnson et al. 1982; Hepper 1983; Heijne et al. 1994), or decrease (Mosse 1973; Chambers et al. 1980; Giovannetti et al. 1981; Buwalda and Goh 1982; Hays et al. 1982; Johnson et al. 1984; Egerton-Warburton and Allen 2000), or have no effect on mycorrhizal colonization (Vázquez et al. 2001). Brown et al. (1981) found that the highest colonization intensities of VAM fungi were in seedling roots grown at a level of N-application optimal for growth, and they found that arbuscule formation was lowest in seedlings grown at the highest N level.

Most previous work on the relationships of VAM fungi and nitrogen has been based either on short-term pot experiments or on short-term field experiments. In this present study, the effects of long-term nitrogen application in field plots are examined.

This study of a semiarid sagebrush community had the following objectives: 1) to determine if the addition of nitrogen or the reduction of nitrogen in experimental field plots would change VAM fungal density associated with plant communities in a semiarid sagebrush ecosystem, and 2) to determine the effect of *B. tectorum* on VAM fungal density.

In order to meet these objectives, field and laboratory studies were designed to examine the change in density of VAM fungi in response to available N levels and plant form. Three field plot treatments were examined: 1) added nitrogen (N+); 2) control (no added nitrogen, C); and 3) sucrose added to reduce nitrogen availability (N-). Two common grasses, *B. tectorum*, an exotic annual weedy grass, and *Agropyron smithii* Rydb., a native perennial grass, were used in this study.

In this work, two hypotheses were tested as possible explanations for changes in community structure:

H_1- The density of VAM fungi would differ between N-amended and sucrose-amended plots in a semiarid sagebrush ecosystem in northwestern Colorado.

H_2- The density of VAM fungi is altered by dominance of *B. tectorum* in a semiarid shrubland ecosystem in northwestern Colorado.

I predicted that the (N+) plots, often dominated by *B. tectorum* plants with the exclusion of a diversity of native plants, would show a decrease in VAM fungal density. And I predicted that the (N-) plots, typically with fewer *B. tectorum* plants and a large number of native perennial plants, would show an increase in VAM fungal density.

2. Materials and Methods

2.1 Site Description

The experimental plots were located in the Piceance Basin of northwestern Colorado, approximately 70 km northwest of Rifle, Colorado, USA, at an elevation of 2020 m. The climate is semiarid with a mean annual precipitation of approximately 280 mm, with half occurring as snowfall. The mean annual temperature is 6.5° C. The soil at the site is fine loamy mixed Borollic Camborthids (McLendon and Redente 1992).

2.2 Experimental Design

A factorial experimental design was used, with four randomized blocks, and each block contained three 160-m^2 treatment plots. Three available-N treatments (N+, C, and N-) were randomly assigned to the three plots in each block. Treatments were begun in 1984 and continue to the present (McLendon and Redente 1992). The low-

N treatment plots (N-) received sucrose, equivalent to 1600 kg C ha^{-1} yr^{-1}, to stimulate microbes to immobilize available N. The high-N treatment plots (N+) received ammonium-nitrate (NH_4-NO_3) at a rate of 100 kg N ha^{-1} yr^{-1}. The intermediate-N treatment plots served as a control (C), and did not receive any soil amendments. The N and sucrose were hand-broadcasted. The N was broadcast in three equal increments annually, and the sucrose in eight equal increments (April through October) in order to provide a more temporally uniform reduction in available N through immobilization.

During the summer season of 1999, mineral soil N availability was monitored in each study plot, and soil and plant root samples were collected. Mineral nitrogen availability was monitored using *in situ* incubations of mixed-bed ion exchange resin (IER) bags (Binkley and Matson 1983). Five IER bags were buried in each plot to a depth of 5 to 10 cm for 6 weeks (from May 21st to July 5th). The buried IER bags adsorb and retain mineral N from the soil solution during the incubation period. Since the bags were exposed to approximately the same environmental conditions as plant roots and microorganisms, the bags provide an index of relative N availability in the different study plots over time. Paschke et al. (2000) noted that IER bags compete with plant roots and microorganisms for available N, so changes in N availability, as detected by IER bags, can result from changes in N-supplying processes as well as changes in plant N uptake.

2.3. Vegetation Biomass and Litter Mass

Aboveground plant biomass, by species, was clipped to ground level twice annually (June and August 1999) in five randomly located 0.5 m^2 quadrats within each of the 12 plots (3 nitrogen treatments x 4 blocks) and oven dried. Plant litter was collected in conjunction with the vegetation sampling. After clipping, the litter was gathered by hand from each of the 0.5-m^2 quadrats.

2.4. Soil Sample Collection, Root Fixation and Staining for Mycorrhiza Inoculum Potential (MIP)

On July 21, 1999, a soil corer (6.5-cm diameter x 15- cm deep) was used to collect ten soil samples from each experimental plot. Five soil cores were centered around the root systems of *B. tectorum* plants, and five soil cores were centered around the root systems of *A. smithii* plants. The soil samples from each plot were placed in a plastic bag, stored in a cooler, and taken to the laboratory for analysis. A sampling depth of 15 cm was chosen because VAM fungal propagule density is generally greatest in the surface 15 cm (Schwab and Reeves 1981; An et al. 1990).

To prepare samples for processing, the larger particles of debris were removed manually, and each soil sample was sieved through a 3-mm mesh screen and then a 1-mm mesh screen to remove roots and stones and to homogenize the soil. The *B. tectorum* or the *A. smithii* roots were removed from the soil and washed gently with tap water and then collected on a 0.5-mm mesh that removed soil particles and retained the small root fragments in order to determine the mycorrhizal status of each species in different plots. The roots were fixed in FAA (formalin, glacial acetic acid, 95% ethyl alcohol, and water 2:1:10:7 v/v/v/v), digested at 90° C for 1 h. in 7 % KOH solution, and stained in 0.05% trypan blue (500 ml glycerol, 50 ml 1% HCl, 0.5 g trypan blue, and 450 ml water) for 10-15 min at 90° C; this is a slight modification of the Phillips and Hayman (1970) method. After staining, excess stain was removed, and the roots were destained in clear acidified glycerin (500 ml glycerol, 450 ml water, and 50 ml 1% HCl). Roots were washed and cut into 1-cm segments. Thirty to forty root segments were randomly selected, placed on a glass slide, covered with a cover slip, and examined with a microscope to detect the presence or absence of colonization by VAM fungi. Only those segments containing mycorrhizal hyphae and either vesicles or arbuscules were counted as colonized. The number of root segment colonized was expressed as a percentage of total root segments in the sample.

After the *B. tectorum* and *A. smithii* roots were removed from the soil cores, the mycorrhizal inoculation potential (MIP) of each soil core sample from each treatment plot was determined using a modification of the Moorman and Reeves (1979) infectivity bioassay. Using this bioassay, one can quantify the relative density of colonization forming units (CFU), which is the relative number of viable propagules of VAM fungi available for colonization of a host plant. CFUs include asexual spores, mycelial fragments, and colonized root fragments. The sieved soil samples were divided into two parts. The first part was placed directly in sterilized 3×20-cm Containers™ (Stuewe and Sons, Inc. Covvallis, OR), and the other part was diluted to 10% (mixed with sterilized sand 1:9, v:v), and placed in 3×20-cm Containers™; soils were diluted to 10% to reduce the number of CFUs in the soil samples. Each 100% and 10% soil dilution was replicated three times; there were a total of 144 Containers™ (3 nitrogen levels x 2 plant species x 4 blocks x 2 dilutions x 3 replicates). Each Container™ was planted with a pregerminated corn kernel (Golden Jubilee hybrid corn, Lilly/Miller Seeds, The Charles Hlilly Co., Portland, OR). Plants were maintained in a growth chamber with a constant temperature of 25° C, and 14 hours light. Plants were watered as needed with tap water. All nutrients except nitrogen and phosphorus (a modified 10% Hogland's solution) were applied at the rate of 50 ml every week. After 30 days, corn plants were harvested, and shoots were cut from roots, and the shoots were dried to constant mass at 55° C and weighed. Roots were washed gently with tap water. The total root volume was determined by the water-displacement method. The roots were fixed and stained for microscopic examination using the procedures described above. Percent root colonization was used to evaluate the changes in numbers of viable VAM fungal propagules to the three nitrogen treatments and plant type (*B. tectorum* and *A. smithii* soils). To assess root colonization, the methods outlined in Rajapakse and Miller (1992) was followed.

2.5. Statistical Analyses

Univariate analyses, using SAS (SAS Institute, Inc., 1989), were used to determine significance. Differences among treatment means were tested using Fisher's least significant difference (LSD) test ($\alpha = 0.05$). The independent variables were nitrogen levels and plant species. Correlation between percent mycorrhizal colonization (MIP) and the dependent variables, total mineral nitrogen availability, total aboveground plant biomass, and relative plant biomass were calculated.

3. Results

3.1. Mycorrhizal Status of *B. tectorum* and *A. smithii*

Roots of *B. tectorum* (an exotic weedy annual species) and *A. smithii* (a native perennial species) collected from the field plots were examined in order to determine whether the roots supported mycorrhizal fungal colonization under the three soils nitrogen levels (N+, C, and N-). Examination of at least 150 root segments for each species showed that both species were mycorrhizal in all plots. The roots collected from the field samples were colonized with hyphae, vesicles, and/or arbuscules of VAM fungi (Figure 2.1). *A. smithii* is known to be a mycorrhizal species (Reeves et al

1979; Allen and Allen 1980); however there is no consensus regarding the mycorrhizal status of *B. tectorum* (Trappe 1981; Pendleton and Smith 1983; Buwalde et al. 1985). Reeves et al. (1979) reported *B. tectorum* to be mycorrhizal in both undisturbed soils and heavily disturbed soils dominated by non-mycorrhizal plant.

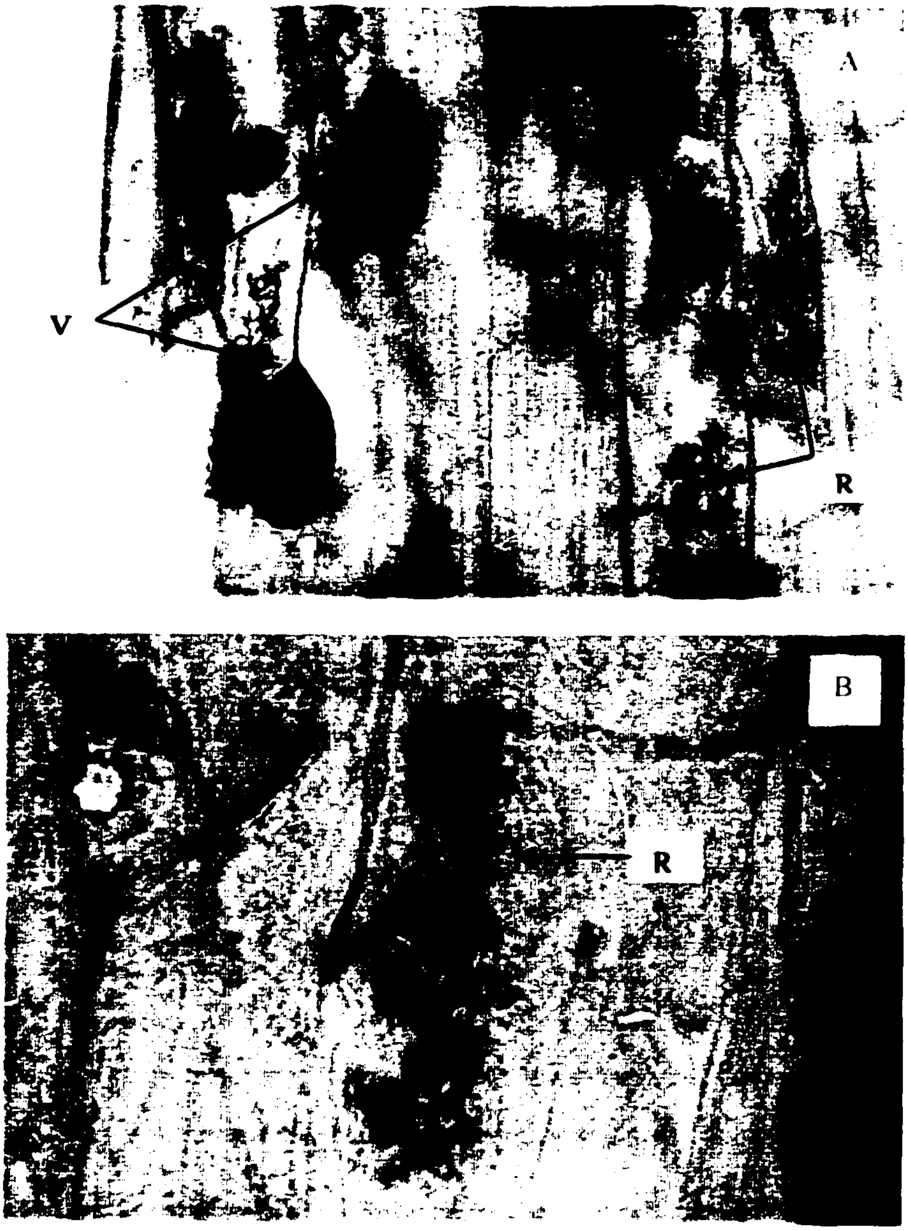

Figure 2.1. Cheatgrass (*Bromus tectorum*) colonized by a vesicular-arbuscular mycorrhizal fungus. A. Overview of root showing both vesicles (V) and arbuscules (R). B. Magnified arbuscule showing the highly branched hyphal ends of the arbuscule.

3.2. Nitrogen Availability, Plant Species and Mycorrhizal Population

Total mineral nitrogen availability as indicated by IER bags, the relative aboveground *B. tectorum* and *A. smithii* biomass, and litter mass data for the three plot treatments are shown in Table 2.1. Correlation of these variables with mycorrhizal inoculum potential (MIP) was performed to determine if there were relationships between these variables and mycorrhizal propagule (spore or colonized root fragment) density at this site. Relative aboveground biomass for *B. tectorum* and *A. smithii* was calculated as follows: [(aboveground *B. tectorum* or *A. smithii* biomass total aboveground plant biomass) X 100] for each experimental plot.

Table 2.1 Means of total mineral nitrogen availability, total aboveground plant biomass, *Bromus tectorum* and *Agropyron smithii* biomass, and litter mass in nitrogen, control, and sucrose plots. Values are overall means ± standard errors from a total of 5 samples per treatment, collected from each of four plots.

Variable	Nitrogen plots (N+) Mean ± SE	Control plots (C) Mean ± SE	Sucrose plots (N-) Mean ± SE
Total mineral N p.p.m	5.40 ± 2.34	2.84 ± 0.21	2.34 ± 0.21
Total aboveground biomass g/m^2	161.30 ±12.5	85.77 ± 18.2	104.14 ± 3.65
B. tectorum biomass g/m^2	22.85 ± 5.18	4.94 ± 2.06	3.84 ± 3.80
A. smithii biomass g/m^2	5.35 ± 4.27	3.15 ± 2.06	1.72 ± 1.13
Litter g/m^2	83.5 0± 17.9	39.49 ± 4.34	73.10 ± 21.0

Both nitrogen availability and plant species are correlated with change in MIP at the two soil concentrations (100 % field soil without any dilution; and 10% soil, field soil diluted to 10% with sterilized sand 1:9, v:v). There were significant differences between N-amended and control plots on MIP with 100% field soil and 10% field soil at $\alpha = 0.05$ (Figure 2.2). The N-addition was associated with decreased MIP in the100% soil with *B. tectorum*: MIP decreased from 89.4% ± 2.16 on the control plots to 68.9% ±4.17 on the nitrogen plots, and in the 10% soil from 30.6% ±4.66 on the control plots to 22.5% ±2.88 on the nitrogen plots. Similarly, there was a general trend but not significant association between N-addition and MIP values. MIP values were reduced on the 100% *A. smithii* soil from 89.7% ±1.90 on the control plots to 82.8% ±3.33 on the nitrogen plots, and on the 10% soil from 50.8%±4.89 on the control plots to 39.7±4.05 on the nitrogen plots.

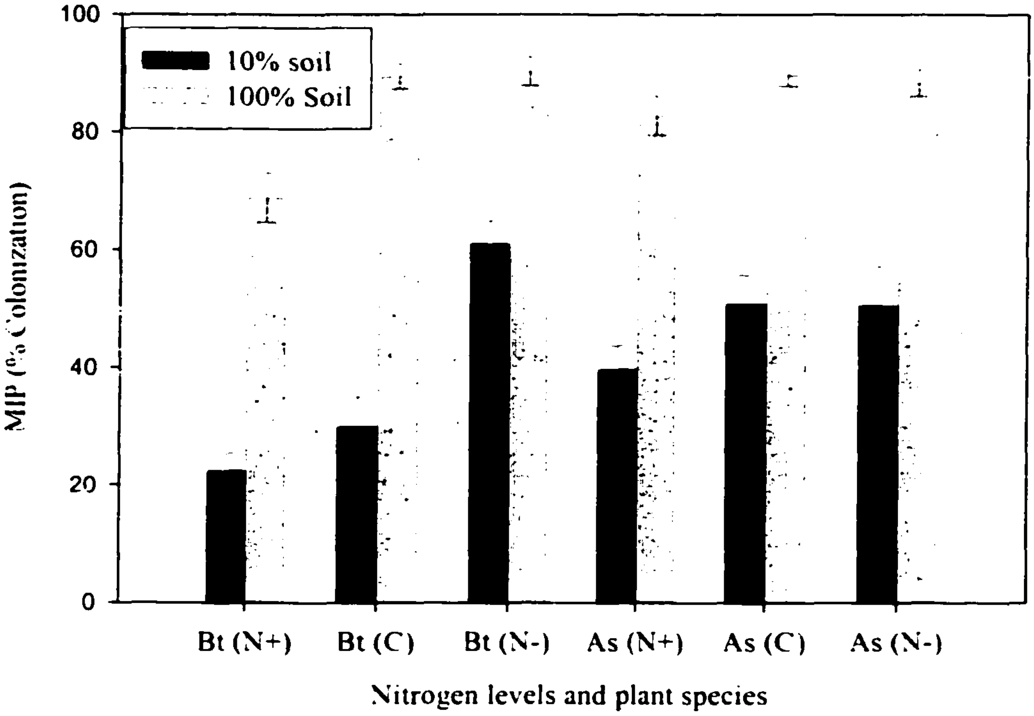

Figure 2. 2. Mycorrhizal Inoculum Potential (as measured by percent root colonization of bioassay maize plants grown in 100% and 10% soils) collected from plots treated at various nitrogen levels and from beneath different grass species. Bt = *Bromus tectorum*, As = *Agropyron smithii*, N+ (nitrogen added), C (control), N- (sucrose added).

In every case, dilution of the 100% field soil with sterile sand to 10% field soil significantly reduced MIP values (Table 2.2). MIP values from 100% and 10% soil from the control (C) and sucrose (N-) plots were not significantly different from each other, except for the comparison between *B. tectorum* in the 10%, control and sucrose plots. The MIP values from on N- plot were significantly greater than MIP values from control (C) plots. On the N+ plots when the *B. tectorum* was a significant component of the aboveground biomass (Table 2.1), the MIP values were consistently lower beneath *B. tectorum* when compared to *A. smithii* (Table 2.2). The interaction between nitrogen levels and species was significant at both 10% and 100% soil concentrations ($F = 4.09$ and $P = 0.021$ in the 100% soil, $F = 9.39$ and $P < 0.0001$ in 10% soil).

Table 2. 2. Means of mycorrhizal inoculum potential (MIP) for 100 % field and 10% field soil (field soil diluted with sterilized soil) collected beneath *Bromus tectorum* and *Agropyron smithii* plants from nitrogen, control, and sucrose plots. Values are overall means ± standard errors from a total of 5 samples per treatment, collected from each of four plots.

Variable	Species	Nitrogen (N+) Mean of MIP ± SE	Control (C) Mean of MIP ± SE	Sucrose (N-) Mean of MIP ± SE
Field soil (100%)	*B. tectorum*	68.89 ± 4.17	89.44 ± 2.16	90.30 ±2.43
	A. smithii	82.78 ± 3.33	89 72 ± 1 90	88.33± 2.27
Diluted soil (10%)	*B tectorum*	22.50 ± 2.88	30.56 ± 4.66	63.06 ± 3.63
	A. smithii	39.72 ±4.05	50.83 ± 4.89	50.56 ± 6.69

Within any treatment (N+, C, or N-) there were no significant differences in shoot weight and root volume of bioassay plants grown in either 100% or 10% soil from *B. tectorum* and *A. smithii*. However, in 100% soil from beneath *B. tectorum* and *A. smithii*, the N+ soil was associated with increased shoot biomass (Figure 2.3, A) and root volume (Figure 2.3, B) of the bioassay plants.

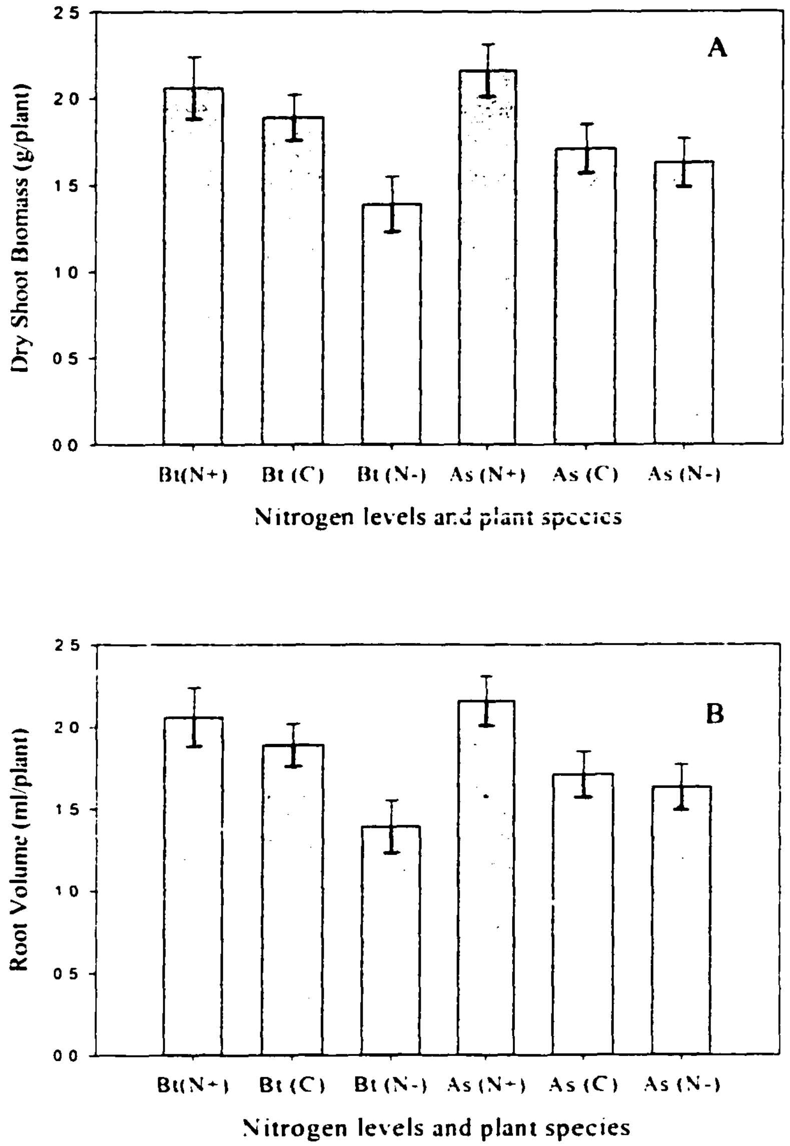

Figure 2.3. Means of (A) dry shoot biomass (B) and root volume of bioassay maize plants grown in field soil (100 %) collected from plots treated with various nitrogen levels and from different grass species. Bt = *Bromus tectorum*, As = *Agropyron smithii*. N+ (nitrogen added), C (control), N- (sucrose added).

3.3. Correlation Coefficients between the MIP and Some Dependent Variables

The correlation coefficients between the MIP values of *B. tectorum* and *A. smithii* soils and other dependent variables (total mineral nitrogen, total aboveground plant biomass, relative aboveground *B. tectorum* plant biomass, relative above-ground *A. smithii* plant biomass, and littermass) indicate some of these properties have the potential to predict MIP and host response (Table 2.3). Results of correlation data from this study indicated a negative relationship (R= -0.63, $P = 0.04$) between MIP of soil from *B. tectorum* and availability of total mineral nitrogen. A strong negative relationship (R= -0.95, $P<0.0001$) between MIP of soil from *B. tectorum* and total aboveground plant biomass, and a negative relationship (R= -0.63, $P = 0.03$) between the MIP values and relative *B. tectorum* aboveground biomass (Table 2.3). In addition, a positive relationship (R= 0.60, $P = 0.04$) between relative *B. tectorum* aboveground plant biomass and total aboveground plant biomass was observed. There also were significant positive correlations between soil total mineral N and relative aboveground biomass for both *B. tectorum* (R= 0.61, $P = 0.03$) and *A. smithii* (R= 0.74, $P = 0.01$).

Table. 2.3. Properties influencing soil mycorrhizal inoculum potential (MIP) beneath *Bromus tectorum* and *Agropyron smithii* plants.

Variable	Partial correlation coefficient (*B. tectorum*)	*P*	Partial correlation coefficient (*A. smithii*)	*P*
Total mineral N	- 0.63*	0.04	- 0.02 ^{NS}	0.96
Total aboveground biomass	- 0.95**	0.00	- 0.33 ^{NS}	0.29
Relative *B. tectorum* biomass (g/m^2)	- 0.63*	0.03	- 0.26 ^{NS}	0.42
Relative *A. smithii* biomass (g/m^2)	- 0.01 ^{NS}	0.98	- 0.21 ^{NS}	0.51
Litter-mass	- 0.46 ^{NS}	0.14	- 0.05 ^{NS}	0.87

*significant correlation at $p \leq 0.05$, ** significant correlation at $p < 0.01$, ^{NS} not significant.

4. Discussion

The results presented in this work support the two hypotheses offered. The density of VAM fungal communities differed between N-amended (N+) and sucrose-amended (N-) plots in the semiarid sagebrush ecosystem and supports the first hypothesis. In N-amended (N+) soil, the VAM fungal densities were lower for *B. tectorum* plants grown in 100% and 10% soil. In the10% in the sucrose- amended (N-) soil the VAM fungal densities were higher. The density of the VAM fungal communities was related to the dominance of *B. tectorum* in this ecosystem. When *B. tectorum* dominates the plots, VAM fungal populations are reduced when compared to plots dominated by native perennial species.

An unexpected result was the MIP value for the soil taken from *B. tectorum* plants on N- plots. MIP was higher than expected and significantly different from other N-treatments. Several explanations for this higher value are possible. The first explanation for this result is based on the observations that the *B. tectorum* plants were rare in N- plots (Table 2.1) and closely associated with perennial. Because the soil samples were collected from directly beneath individuals of each plant species in the field study, there were some variations in species composition of neighboring plants among the samples. Thus, it was not possible to completely exclude rhizosphere soil and roots of competing species due to the complex belowground root networks. The high MIP value beneath the sparsely-spaced *B. tectorum* plants in the N- plots may be due to the presence of roots (mycorrhizae) of other species. Moreover, with low N availability individual plants may develop a wide depletion zone around their roots, thereby reducing the availability of nutrients for adjacent plant roots in the same soil volume. Such plants will, therefore, experience relatively high competition intensity (Ozinga et al. 1997). The second possible explanation for increased MIP values is that the bioassay corn plants under reduced nutrient conditions might benefit from VAM fungi and thus exhibit increased root colonization. The third possible reason for increased mycorrhizal colonization in roots of a bioassay host grown in 10 % soils beneath *B. tectorum* on N- plots is due to increased nutrient stresses. In the 10% soil available N, P and other elements are reduced approximate 90% by sterilized sand, the nutrient solution lacks both N and P, and the original field soil may lack nutrients due to *B. tectorum* plants. Thus the corn bioassay plants were severely stressed for essential minerals. The effect appears quantitative that greater the number of *B. tectorum* plants the greater effect on VAM fungal population. A single *B. tectorum* plant has limited effects on VAM fungi because of the effects of other native plant species.

Despite the reduction of MIP by increasing of N+, roots of both species in general showed higher MIP values on N+ plots. This may be because the phosphorus concentration in the soil was lower than the critical level. A large demand for P may stimulate mycorrhizal colonization (Smith and Read 1997). Hepper (1983) suggested that P:N is the most important factor affecting the mycorrhizal root colonization. Bentivenga and Hetrick (1992) reported that N fertilization increased P demand and demand for mycorrhizae. Also this would explain the often non-significant reduction of the level of MIP values in *A. smithii* soils on N+. The high level of colonization by VAM fungi in plant roots corresponded to periods of P demand in the plants (Fitter 1991).

The VAM fungal populations did not change significantly with different nitrogen availability in the soil beneath *A. smithii* plants, while they decreased in *B. tectorum* soils. Nitrogen fertilizations can change plant community composition (Tilman 1986). Klein et al. (1989) reported that the change in a plant community due to a change in nitrogen availability is reflected in changes in rhizosphere processes. They hypothesized that most of these changes might occur in earlier successional species. Because of this the early successional plants, such as *B. tectorum*, show a greater response to fertilization than occurs in later successional plants, such as *A. smithii*. Since *A. smithii* is a later successional plant, the VAM fungal populations did not show a high reduction beneath *A. smithii* plants on N+ plots. Chapin (1980) reported that soil nutrient variations can have a lower effect on the later succession species than earlier succession species. Since VAM fungi are important components of rhizosphere processes, changes in VAM fungal population would lead to significant changes in over all soil processes. *B. tectorum* significantly changes VAM fungi populations. Thus, the invasion of *B. tectorum* would be expected to change important soil processes.

Findings of reduced VAM fungal populations in plots with elevated nitrogen availability concur with results reported by others (Brown et al. 1981; Giovanetti et al. 1981; Bledsoe and Zasoski 1983; Johnson et al. 1984; Baltruschat and Dehne 1989; Ellis et al. 1992; Miller and Jackson 1998; Hawkins and George 2001). Giovanetti et al. (1981) and Johnson et al. (1984) found that mycorrhizal formation was decreased or inhibited by high N level. Bledsoe and Zasoski (1983) noted that adding nitrate-N at a level of 100 ppm in the soil decreased ectomycorrhizal colonization on Douglas-fir seedlings. The highest colonization intensity were found by Brown et al. (1981) in root samples from seedlings grown at N-application levels optimum for growth (280 - 560 kg N h^{-1}), and arbuscule formation was poorest in seedlings grown at the highest N level (1,120 - 2,240 kg N h^{-1}). Egerton-Warburton and Allen (2000) found that an increasing input of nitrogen was associated with the displacement of the larger-spored species of *Scutellospora* and *Gigaspora*, due to a failure to sporulate, with a concomitant proliferation of small-spored *Glomus* species (e.g. *Glomus aggregatum* and *Glomus leptotichum*). Also they reported that nitrogen availability also significantly reduced spore abundance, modified the timing of VAM spore production in most sites they examined, and reduced hyphal and vesicular root infection. Douds and Schenck (1990) found that sporulation is decreased by P fertilization in many VAM species, and also by N fertilization in some species. On the other hand, Hawkins and George (2001) attributed the decreased mycorrhizal colonization with increasing nitrogen availability to an increased P absorption in some plants.

In contrast, many researchers found positive relationships between N availability and VAM fungal colonization. Hepper (1983) reported increased colonization of lettuce roots by the VAM fungus *Glomus mosseae* at three different P levels with increasing NO_3^- concentration. Onguene and Habte (1995) observed a positive relationship between nitrogen amount in soil solution and root mycorrhizal colonization of *Leucaena leucoephala* seedlings. Aziz and Habte (1989) also found increased root colonization of *Leucaena* by the VAM fungus *Glomus aggregatum* with application of NH_4-NO_3. Eom et al. (1998 and 1999) noted that nitrogen fertilization significantly increased root colonization and extraradical mycorrhizal hyphae development but did not affect the VAM spore abundance and fungal species diversity or richness. Verkade and Hamilton (1983), Furlan and Bernier-Cardou (1989), and Dhillion and Ampornpan (1992) reported that nitrogen fertilization stimulates root colonization by VAM fungi. On the other hand, Buwalda and Goh (1982), Coxwell and Johnson (1985), and Bååth and Spoken (1989) found decreases in infection rate, whether they added ammonium or nitrate.

These results and others have shown that N fertilization suppressed the root colonization by VAM fungi at least with *B. tectorum*, and contradict the findings of some other studies. There could be several explanations for the contradictory responses of VAM fungi colonization to nitrogen availability in different studies, including differences in rhizosphere soil characteristics. As indicated by Hepper (1983) it is important to consider the ratio of applied nitrogen to phosphorus (Bååth and Spoken 1989; Guttay and Dandurand 1989; Sylvia and Neal 1990; Onguene and Habte 1995; Miller and Jackson 1998). Also, many previous studies indicated that the type of response by plants to VAM fungi colonization is dependent on the host plant species (Miller and Jackson 1998; Harinikumar and Bagyaraj 1989), different strains of mycorrhizal fungus species (Boucher et al.1999), and prevailing environmental conditions (Allen 1991). Environmental conditions that affect plant and mycorrhizal growth such as temperature (Hayman 1974), light intensity (Hayman 1974; Johnson et al. 1982), moisture (Ellis et al. 1992), and soil type (Brady 1990; Anderson et al. 1994; Frey and Ellis 1997) could affect VAM fungi colonization function. The various amounts of ammonium-N and nitrate-N needed by plants for satisfactory growth (Chambers et al. 1980; Azcon et al. 1982; Hays et al.1982; Vaast and Zasoski 1992; Heijne et al. 1994), and frequent additions of fertilizers to plants (Douds and Schenck 1990) could affect the mycorrhizal plant relationship.

Reduced nitrogen availability was associated with increased mycorrhizal density in the soil. Sucrose amendments to the plots have been shown to speed-up plant succession by changing the plant community composition from annual weedy species to perennial species (McLendon and Redente 1992; Paschke et al. 2000). My results indicate that elevated nitrogen availability is associated with reduced VAM fungal density in soil beneath *B. tectorum*. This decrease may be due to increasing those annual weedy species such as *B. tectorum* with dependency on a fewer number of VAM fungi and decreasing aboveground plant diversity. Bentivenga and Hetrick

(1992) and Paschke et al. (2000) found that nitrogen amendments resulted in a significant increase in the forbs and the persistence of weedy annual species, while the cover of C_4 grasses decreased. When the number of different potential host plants decrease due to an increase in nitrogen availability, the VAM fungal density will decrease. Johnson et al. (1991) found that species composition of VAM fungal communities changed during secondary succession. They also noted that late successional stages had a greater dependence on mycorrhizae in terms of growth benefit from colonization, while those of intermediate stages have little dependence, and mycorrhizal fungi negatively impact the non-mycorrhizal annuals of the first stages of succession. They also suggested that the rate of succession might be related to the increase of mycorrhizae with time. In the nitrogen addition (N+) plots, annual grasses and annual forbs strongly dominated the community, and the plots had few perennials (Paschke et al. 2000). Because some annual plants are less dependent on VAM fungi, the numbers of viable VAM fungal propagules are likely to be less. Also, as the number of plant species decrease, the number of VAM fungal species decreases (van der Heijden et al. 1998). Bentivenga and Hetrick (1992) found that when nitrogen increases, forbs increase, but the cover of C_4 grasses decrease, and *Glomales* spore populations decrease. Thus, changes in *Glomales* spore populations in response to N fertilization may be related to changes in host populations. Even weed dynamics in agriculture fields have been associated with changes in VAM spore populations (Kurle and Pfleger 1994).

Results from this study enhance our understanding of VAM fungi and support the concept that VAM fungi are an important factor in the establishment of late-seral plant species. McLendon and Redente (1992) found that the addition of sucrose to these same study plots in a semiarid sagebrush community reduced exotic early-seral annual weeds. This may be due to increasing mycorrhizal density associated with reduced nitrogen availability, resulting in late-seral mycorrhizal plant species out-competing the early-seral weedy annual species that are typically non-mycorrhizal.

5. Conclusions

The present study gives a general view of the mycorrhizal status of *B. tectorum* in plots with varying N availability in a semiarid sagebrush community. The ability of *B. tectorum* to compete under conditions of high soil N may explain why this grass has been so successful and dominates the nitrogen enriched plots. These results may shed light on the N dynamics of mycorrhizal native plants in the sagebrush community.

The results of these experiments indicate there is a great potential for predicting mycorrhizal density responses to both increasing nitrogen availability and plant type. Increasing soil nitrogen seems to reduce beneficial interactions between VAM fungi and these grasses. According to the cost-benefit model developed by Fitter (1991), mycorrhizal colonization is beneficial to a plant when phosphorus is growth limiting. This might be true for nitrogen and mycorrhiza as well. Sylvia and Neal (1990) also suggested that plant nitrogen demand, like plant phosphorus demand, promotes root colonization by VAM fungi. However, nitrogen treatments produced the greatest biomass for these plant species, and this suggests that nitrogen may have decreased plant dependency of VAM fungi and resulted in a decrease in VAM fungal colonization. Since root volume of bioassay host plants significantly increases in soil from N+ plots, the increased root volume could increase P uptake as well.

Beneficial effects such as reduced need for fertilization, increased seedling survival in the field, and tolerance to harsh conditions following establishment of mycorrhizal symbiosis can be more important for range plants than better growth resulting from nitrogen addition. Finally, adequate amounts of nutrients for rapid and vigorous early growth and maintaining nutrient levels low enough to promote maximal colonization of roots by VA mycorrhizal fungi may be best for seedling establishment, growth, and survival. In an efficient rangeland management program, mineral fertilizer applications should be low enough to avoid undesired suppressions of mycorrhizal formation. Effects on VAM symbiosis and community structure are important because VAM fungi influence the growth, competitive relationships, and abundance, diversity and community structures of plants in rangelands.

Chapter 3

Effect of Cheatgrass -*Bromus tectorum* L.- on Vesicular-Arbuscular Mycorrhizal Populations at Disturbed Sites

1. Introduction

The roots of most terrestrial plants form symbiotic associations with vesicular-arbuscular mycorrhizal (VAM) fungi. These fungi colonize the roots of approximately 90-95% of all vascular plant species (Gerdemann 1968; Hayman 1981; Sylvia and Williams 1992) and can contribute to plant growth and survival by reducing stress (Francis and Read 1992; Sylvia and Williams 1992). They improve plant growth and yield, increase nutrient and water uptake, and suppress plant diseases (Schenck 1981; Hayman 1983). Infectivity of VAM fungal propagules and mycorrhiza effectiveness can be influenced by abiotic and biotic differences among habitats (Allen 1991; Brundrett 1991).

Following disturbance, annual or biennial ruderal plant species often invade. Many of these weeds can complete their life cycle in the brief period of time between a disturbance event and recolonization by perennial species. These weeds may be either nonmycorrhizal or mycorrhizal species such as cheatgrass (*Bromus tectorum* L.). In contrast to many ruderal annual species, most perennial species are mycorrhizal (Francis and Read 1992; Wicklow-Howard 1994).

B. tectorum was introduced into the North America continent from the Mediterranean area of Europe, and it is considered a major weed problem in United State rangelands (Nesse and Ball 1994). *B. tectorum* is adapted to an annual precipitation of 150 to 550 mm, and can colonize a wide range of soil conditions (Billings 1994; Pyke and Novak 1994). *B. tectorum* also is able to obtain moisture more effectively than native bunchgrasses because of its root phenology and rate of root growth, and thus inhibits the establishment of native perennial bunchgrasses and impedes successional change (Harris 1977).

Miller (1979), Reeves et al. (1979), Janos (1980), and Wicklow-Howard (1994) hypothesized that, under disturbed soil conditions where mycorrhizae are reduced, secondary succession will be slowed, resulting in prolonged longevity of early successional non-mycorrhizal plants. Allen and Allen (1980) suggested that the rate of plant succession on a disturbed site might be dependent on the rate at which VAM fungi increase with time. Wicklow-Howard (1994) summarized the impacts that land disturbance may have on mycorrhizae. She suggested that disturbance lowers mycorrhizae abundance, increases nutrients available to plants, and leads to the dominance of nonmycorrhizal species in early stages of succession. Success of nonmycorrhizal species further reduces the propagules of mycorrhizal fungi and succession can be slowed because of the lack mycorrhizal fungi (Reeves et al. 1979; Reeves and Redente 1991). The increased dependency of plants on mycorrhizal symbiosis varies, but for plants on undisturbed sites the relationship is considered important (Linderman and Pauliz 1990).

Plant nutritional demands vary among species. If proper and sufficient levels of nutrients are available, many plants will grow normally without mycorrhizal fungi. However, without adequate amounts of essential elements the plants will grow poorly or not at all (Stubblefield et al. 1987). In the presence of VAM fungi, the competitive ability of mycorrhizal plants is enhanced when certain nutrients are limiting, whereas that of nonmycorrhizal plants is reduced so long as the plants are competing for the same limited soil resources (Reeves 1985). The inhibition of successional change by *B. tectorum* suggests this taxon retains its competitive advantage. *B. tectorum* may derive more benefit from the symbiosis than do the competing native grasses (Buwalda et al. 1985).

Based on literature reviews, Trappe (1981) and Pendelton and Smith (1983) noted that *B. tectorum* is a facultative host of VAM fungi. They reported that when *B. tectorum* grows with non-mycorrhizal plants, it is nonmycorrhizal, whereas when it grows in the presence of mycorrhizal plants, it is mycorrhizal. On the other hand, Reeves et al. (1979) reported *B. tectorum* to be mycorrhizal on both heavily disturbed soils characterized by predominantly non-mycorrhizal species and on undisturbed soil.

Plants with highly branched root systems may not necessarily derive as great a benefit from the mycorrhizal symbiosis as plants with less developed or sparse root systems (Hayman 1983; Koide and Li 1991b). Goodwin (1992) pointed out that *B. tectorum*, with a highly developed root system, sustains root growth in lower, warmer soil horizons during winter when native grasses are dormant, and may benefit from mycorrhizal colonization from the time of germination. Also the rapid growth rate of *B. tectorum* provides a strong sink for water and nutrients taken up by VAM fungi.

Host plants are the most important factor that regulates VAM fungal populations and species composition (Hetrick and Bloom 1986; Sanders and Fitter 1992; Bever et al. 1996, van der Heijden et al. 1998). The VAM fungal composition and density can be influenced directly by host plants by regulating carbon allocation to the roots, producing secondary metabolites or by changing soil environmental conditions (Eom 1998). Mycorrhizal fungi associated with plant species may change as vegetation develops following disturbance (Helm et al. 1996). Johnson et al. (1992) and van der Heijden et al. (1998) also noted that changes in host plant community structure can affect VAM fungal species composition, so different plant hosts can influence changes in the mycorrhizal fungal population in the root zone.

Species of mycorrhizal fungal communities has been correlated with the species richness of plant communities in temperate grasslands (Johnson et al. 1991; van der Heijden et al.1998). Depending on plant species, long-term continuous dominance of a plant community may increase or decrease VAM fungal colonization and spores numbers (Black and Tinker 1979; Harinikumar and Bagyaraj 1988). Harinikumar and Bagyaraj (1988) found that growing a nonmycorrhizal plant in soil for one season reduced mycorrhizal colonization of subsequent plants by about 13%, and a fallow period reduced colonization by 40%. Grass invasion by new mycorrhizal species also could cause a parallel decline in the species richness of mycorrhizal fungal communities (Johnson and Wedin 1997).

Soil disturbance often causes severe decline in residual propagules of indigenous mycorrhizal fungi. It has been suggested that re-establishment of vegetation on disturbed sites depends on management practices that enhance mycorrhizal formation (Warmer 1983; Visser et al. 1984; Stahl et al. 1988; Cuenca and Lovera 1992; Janos 1996). The success of such management practices depends on understanding the population dynamics of the mycorrhiza inoculum in the soil (changes in the mycorrhiza inoculum potential) and the extent to which plant performance is affected by mycorrhiza formation (Bakarr 1997).

Assessment of VAM fungal density and diversity in soil is a crucial step towards developing a starting point for effective management of indigenous fungal populations (Allen 1991; Janos 1996). Study approaches to assess VAM fungi differ from those used to study other microorganisms because of the fundamental differences in the biology of mycorrhizal growth and development. Since VAM fungi cannot be grown alone in culture, they are studied either directly, via counts and identifications of spores in soil, or by inference, using roots that are colonized. There are three basic methods to estimate the number of colony forming units (CFUs), viz., Most Probale Number (MPN), Mycorrhizal Inoculum Potential (MIP), and Spore Counting (SC). Molecular methods are increasingly useful for assessing fungal diversity associated with root segments, as are staining methods used to document and quantify root colonization.

Because many soils are heterogeneous, the exact numbers of an individual organism can be impractical to determine. Quantification studies of VAM fungi are, by nature, bioassays of propagules, and relative numbers of viable propagules are determined by planting an assay host on dilutions of the soil followed by qualitative evaluation for endogonaceous colonization and quantitative analysis by Mycorrhizal Inoculum Potential (MIP) or Most Probale Number (MPN) assays. In general, infective propagules that are recognized as components of VAM fungi inoculum include asexual spores, hyphae in living root fragments, hyphae in dead root and other organic matter, and intact hyphal networks attached to or detached from roots (Alexander 1982; Tommerup 1992; Janos 1996). It is difficult to determine the contribution of mycorrhizal fragments to mycorrhizal inoculum potential in field soils, because such soils can contain mixtures of propagule types. Mycorrhizal fragments may be most important when the soil lacks spores; severing roots during soil extraction for bioassay produces mycorrhizal fragments that may lead to greater propagule density estimates. Bakarr (1997) mentioned that vesicles might function as spores, especially in roots that are senescing.

VAM fungal spores are the swollen structures with one or more subtending hyphae that form in the soil or in roots. Spores usually develop thick walls, which often have more than one layer. They can function as propagules. VAM fungal spores are one source of mycorrhizal colonization of plant roots (Baum and Makeschin 2000), and spore counting (SC) is one of the methods for measuring propagule numbers. The method involves a direct count of spores extracted from soil samples. Counting spores in the soil depends on wet sieving and decanting, either alone or in conjunction with other separation techniques. In this method the soil is suspended in water and passed through a series of sieves arranged in decreasing order of mesh size. Although spore count is one of the most common methods for quantifying VAM fungi inoculum in the soil, it is indecisive due to the effects of plant nutrients, season, host plant, and possibly other factors on the production of spores (Moorman and Reeves 1979).

The most probale number (MPN) technique is an important technique in estimating microbial populations in soils, waters, and agricultural products. This method has been used for estimating the numbers of infective propagules of symbiotic organisms and soil-borne plant pathogens (Porter 1979; Wilson and Trinick 1982; Woomer 1994). The MPN method measures presence or absence of mycorrhizal fungi in a dilution series, with the results interpreted as a probability estimate of propagule number from a statistical table. MPN provides a relative measure of the density of propagules capable of colonizing roots. Wilson and Trinick (1982) mentioned that MPN could provide very useful data on the colonizing capacity of VAM fungi. Also, they pointed out that estimating propagule numbers is strongly dependent on the conditions used, and the values are relative, not absolute.

The mycorrhizal inoculum potential (MIP), defined as the capacity of infective propagules of VAM fungi existing at a site or micro-site (particular soil) to form sufficient mycorrhizae (Janos 1996; Bakarr 1997), is crucial to understanding the potential importance of mycorrhizae in the field. MIP is a product of the abundance and vigor of the propagules in the soil and can be quantified by determining the rate of colonization of a susceptible host under a standard set of conditions (Moorman and Reeves 1979). Techniques for assessment of MIP are based on soil extraction and manipulation, both of which fragment roots and usually exclude extensive hyphal networks. Among the many factors that affect the estimation of propagule numbers and inoculum potential are: number of propagules present, the rate of root growth and pattern of branching, environmental variables that control plant and fungal growth, soil nutrient levels, and size of propagules. When these factors are not normalized or taken into account results are likely to be unreliable.

The effects of weed invasion on mycorrhizal density in grasslands have not been well studied. Researchers have employed only one of these methods, and a few studies have utilized two of these methods The objectives of this study were to determine the effects of *B. tectorum* on VAM fungal population density as measured by three traditional methods, namely MIP, MPN, and SC in two sites on Colorado, and to determine how these traditional methods used to quantify the relative densities of colonizing propagules of VA mycorrhizal fungi compare, correlate, and/or differ.

In these experiments, I tested the hypothesis that VAM fungal population density would be affected significantly by presence of *B. tectorum*, and I predicted that the three different methods would give similar results in term of VAM fungal population densities. I expected that the VAM fungal population density would be reduced when *B. tectorum* dominates a site.

2. Materials and Methods

2.1. Study Sites

Soil samples were collected from two sites in Colorado. The first location was an old-field (shortgrass steppe) near the Central Plains Experimental Range (CPER) located 50-km northeast of Fort Collins, Colorado. Elevation of the CPER is approximately 1950 m, and the annual precipitation is approximately 310 mm, with 70% occurring during the growing season. The last cultivation of this area was in 1981, and the soil at this site is an Ascolon fine sandy loam. Vegetation at this site is dominated by *B. tectorum* L., *Agropyron desertorum* (Fisch. ex Link), and *Sporobolus cryptandrus* (Torr.) A.Gray (Paschke et al. 2000). The second location was in the Piceance Basin of northwestern Colorado (sagebrush steppe) (see Chapter 2 for a description of this location).

2.2. Soil Samples Collection

On May 12, 2000, three soil samples within each replication (each core 6.5-cm diam x 15-cm deep) were collected near the CPER beneath *B. tectorum* plants that were isolated from other grasses and present in large patches. Three similar samples were collected near the CPER from patches without *B. tectorum* but with other species present. On July 21, 2000, three soil samples were collected from the *B. tectorum* patches in Piceance Basin that were dominated with *B. tectorum* but also had some other species within the patches. In addition, three soil samples were also collected from the control patches that contained no *B. tectorum* plants. Each sample was placed in a plastic bag, placed in a cooler, and taken to the laboratory for analysis. Each sample was mixed and divided into three portions: the first part was used for analysis of spore numbers, the second part was used for a MIP bioassay, and the third part was used for MPN bioassay. These soil samples were examined for mycorrhizal fungal density by counting spores directly and by growing bioassay plants (*Zea mays* L.). The fungal densities were determined in order to detect if there was any significant effect of the presence of *B. tectorum* on mycorrhizal colonization and mycorrhizal spore numbers.

2.3. Determination of Density of VAM Population

2.3.1. Most Probale Number (MPN)

Large particles of organic debris (leaf and stems pieces) were removed manually, and each soil sample was sieved through a 3-mm mesh screen and then a 2-mm-mesh screen to remove stones and to homogenize the soil. A portion of soil of each replicate was diluted 10-fold using sterilized washed sand. Dilutions up to 10^{-4} (100%, 10%, 1%, 0.1% and 0.01%) (0:140, 126:14, 138.6:1.4, 139.86:0.14 and 139.986:0.014 sand:field soil v:v respectively) were prepared by mixing field soil and sterilized sand in plastic bags. Field soil (100%) and diluted soil were placed in disinfected 3×20-cm Containers™, replicated five times, and then each was planted with a pregerminated corn kernel (Golden Jubilee hybrid corn, Lilly/Miller Seeds, The Charles Hlilly Co., Portland, OR). Corn kernels were washed several times with water, placed in sterilized moist germination paper, and kept in a growth chamber at 25°C for 48 h. One healthy germinated corn kernel was carefully transplanted to each Container™ and covered with sterilized sand. Plants were kept under growth chamber conditions with temperature set at 25°C, the light set at 14 hours day and 10 hours night, and plants were watered as needed with tap water. All nutrients except phosphorus (a modified 10% Hoagland's solution) were applied at the rate of 50 ml every week. The bioassay plants were grown for four weeks until harvest. After separation from the shoot each entire root system was washed gently with tap-water, fixed in FAA (Formalin, glacial acetic acid, 95% ethyl alcohol, and water, 2:1:10:7 v/v/v/v), stored at room temperature for at least 48 hours, and stained with 0.05% trypan blue (500 ml glycerol, 50 ml 1% HCl, 0.5 g trypan blue, and 450 ml water) (Rajapakse and Miller 1992) at 90° C for 10-15 min. After staining, excess stain was removed, and the roots were destained in clear acidified glycerine (500ml glycerol, 450ml water, and 50 ml 1% HCl. Each root system was examined with a dissecting microscope at 20-60x to detect the presence or absence of colonization. A root system was considered positive for VAM fungi if it contained typical aseptate internal hyphae, and arbuscules or vesicles. The presence of detached hyphae or spores was not counted in scoring procedures. The MPN value is determined from counts of positive tubes (those root systems with mycorrhizal colonization) in different dilutions. The first dilution to be measured is the lowest one with the maximum number of positive tubes, 5 in an assay with 5 tubes per dilution, along with the next three dilutions. The number of positive tubes in each of these four dilutions is applied to a table of most probable numbers, and confidence intervals are calculated by using a confidence factor table (Woomer 1994). The lower confidence limit at ($p = 0.05$) was calculated by dividing the population estimate by the confidence factor. Similarly, the upper limit results from multiplying the population estimate by the confidence factor (Woomer 1994).

2.3.2. Mycorrhizal Inoculum Potential (MIP)

A modification of Moorman and Reeves (1979) infectivity bioassay was used to quantify the relative density of colonizing propagules of VAM fungi (Chapter 2). In this experiment the field soils were diluted with sterile sand to five concentrations, 1^0,

1^{-1}, 1^{-2}, 1^{-3}, and 1^{-4}. The same procedures used to determine mycorrhizal density by MPN was used. The difference between the two methods is that in the MIP assay the root systems of bioassay plants were washed and cut into 1-cm segments. Forty to fifty segments were randomly selected, placed on a glass slide, covered with a cover slip, and examined with a compound microscope at 40x to detect the presence or absence of colonization, instead of examining the entire root system of bioassay plants. After examining roots for the presence or absence of VAM structures, the number of root segments colonized by VAM fungi was counted and expressed as a percentage of total roots segments in the sample. The percentage colonization was calculated as the number of segments with any colonization out of the sample of 100. Only those segments containing mycorrhizal hyphae and either vesicles or arbuscules were counted as colonized.

2.3.3. Spore Counts (SC)

To examine spore population density in soils, standard wet sieving and decanting methods (Pacioni 1992) were followed in each soil sample. The soil samples were sieved through a 3-mm mesh screen and then a 2-mm mesh screen to remove roots and stones and to homogenize the soil. Spores were extracted from 2.5-ml soil sub-samples by wet sieving. Each 2.5-ml soil sample was suspended in 150-ml water, stirred for 10 min with a magnetic stirrer, the heavier particles were allowed to settle for 15 sec, and the suspension was passed through a three-sieve series consisting of 500, 106 and 45μm mesh. The contents of each sieve were transferred by washing to a 30- ml polyethylene centrifuge tube and were centrifuged for 3 min at 1270X g (3000 RPM in a clinical centrifuge with a 12-cm radius head) to settle the soil particles. VAM fungal spores float and adhere to the sides of the centrifuge tube.

Immediately spores were rinsed out of the centrifuge tube three times to ensure removal of most of the spores. Each rinse was collected on Whatman™ Grade 8-ruled filter paper in a Buchner funnel by vacuum filtration. The filter paper and spores were transferred to 10-cm petri dishes to count the spores by means of a dissecting microscope. Total spore counts were expressed as number of spores per gram dry soil. Soil dry weight was determined by weighing 2.5-ml sub-samples of each soil and dried at 95° C for 24 hrs, and reweighting the soil (Johnson at el. 1991).

2.4. Statistical Analysis

A one-way analysis of variance (ANOVA) was performed using SAS (SAS Institute, Inc., 1989) to evaluate the effect of the annual weed, *B. tectorum*, on VA mycorrhizal fungi population based on mycorrhizal inoculum potential (MIP), most probable number (MPN), and total spore numbers (SC) of each soil sample. Correlations between the three procedures were made to determine the relationships among these study methods. The MPN data were transformed to $\log_{10}$ because the logarithmic distribution tends to be more symmetrical than the distribution of the estimated density (Cochran 1950). Means of the five replicates for each main sample were used in correlation analyses.

3. Results

3.1. Effect of *B. tectorum* on VAM Population Estimated by Three Different Methods

3.1.1. Most Probale Number (MPN) Estimates

Table (3.1) shows that the number of propagules of VAM fungi in the abandoned cropland (the CPER site) are significantly lower beneath *B. tectorum* (one-way ANOVA: F = 7.85, $p = 0.049$) when compared to the control. The absolute number of VAM fungal propagules in the soil collected beneath *B. tectorum* plants, as measured by the MPN method for this site, was reduced compared to soil collected from patches without this weed but with other perennial species present (Control). The mean (± SE) propagule numbers were 40.20 ± 21.4 for the soil from *B. tectorum* and 734.20 ± 247 for the control soil.

In Piceance Basin soil, there was a general trend but not a significant reduction in the MPN of VAM fungi by *B. tectorum* (one-way ANOVA: $F = 1.45$, $p = 0.294$). The presence of *B. tectorum* reduced the mean MPN of the VAM fungal density from 269 ± 58.7 in soil samples beneath perennial plant species (control) to 186.59 ± 35.7 in the soil beneath *B. tectorum*.

Table. 3.1. Number of infective VAM fungal propagules (± standard error) estimated by using two methods- the MPN and wet-sieving spore count method - in two soils, collected from patches dominated by *Bromus tectorum* plants and patches without *B tectorum* plants (Control) in the Central Plains Experimental Range (CPER) and Piceance Basin of northwestern Colorado.

Location	Soil collected from beneath	MPN ± SE (Propagule cm^{-3} soi)	Spore Count ± SE (Spore g^{-1} soil)
CPER	*B. tectorum*	40.20^{B*} ±21.4	24.01^{b} ± 2.38
	Control	734.20^{A} ±247	51.40^{a} ± 7.33
Piceance	*B. tectorum*	186.59^{Z} ±35.7	100.69^{z} ± 22.1
	Control	269.46^{Z} ±58.7	102.34^{z} ± 8.72

* Means followed by same letter in a column do not differ at $p = 0.05$ (LSD).

3.1.2. Mycorrhizal Inoculum Potential (MIP) Estimates

The MIP values (percentage colonization) in the soil samples collected beneath *B. tectorum* plants and patches without *B. tectorum* plants on the CPER were significantly different (Table 3.2, figure 3.1 A). *B. tectorum* plants that colonized after crop cultivation was abandoned on this site were associated with lower VAM fungal density. When soil from *B. tectorum* plants was compared to soil without this weed, the MIP values were significantly lower in all *B. tectorum* soil dilutions. In *B. tectorum* soils, the mean MIP values (± SE) were 16.44% ± 3.45, 5.78% ± 1.44, 1.11% ± 0.42, 0.22% ± 0.22 in 1^0, 1^{-1}, 1^{-2}, and 1^{-3} in diluted soils respectively. But in control soil the MIP values were 78.89% ± 2.75, 34.22% ± 2.44, 7.78% ± 1.37, 3.33% ± 0.65 in 1^0, 1^{-1}, 1^{-2}, and 1^{-3} diluted soils respectively. No VAM fungal colonized was found in 1^{-4} diluted soils from either soil source (Table 3.2 and Figure 3.1).

For the Piceance Basin soils, I found only the 100% field soil showed a significant difference in VAM fungi when the % MIP values collected from *B. tectorum* plants and the control soil samples were compared. Mean MIP value (± SE) in soil (1^0) was 78.00% ± 4.42 for *B. tectorum*, while it was 93.11% ±1.54 for soil without *B. tectorum*. No significant differences were found in the other soil dilutions but there was a general trend of reduced MIP values for *B. tectorum* in the diluted soils compared to control soil at all dilutions (Table 3.2 and Figure 3.1). In every case, the MIP values in soils from CPER were less than the MIP values in soils from Piceance Basin in both *B. tectorum* and control patches (Table 3.2.).

Table. 3.2. Mycorrhizal Inoculum Potential (± standard error) as measured by percent root colonization of bioassay maize plants grown in different soil dilutions collected from patches dominated by *Bromus tectorum* plants and patches without *B. tectorum* plants (Control) in the Central Plains Experimental Range (CPER) and Piceance Basin of northwestern Colorado.

Location	Soil source	Soil dilutions (%)				
		100	10	1	0.1	0.01
CPER	*B. tectorum*	16.44±3.45	5.78±1.44	1.11±0.42	0.22±0.22	0±0
	Control	78.89±2.75	34.22±2.44	7.78±1.37	3.33±0.65	0±0
Piceance	*B. tectorum*	78.00±4.42	36.89±2.90	10.67±1.60	0.22±0.22	0±0
	Control	93.11±1.54	42.44±2.77	14.89±2.27	0.44±0.44	0±0

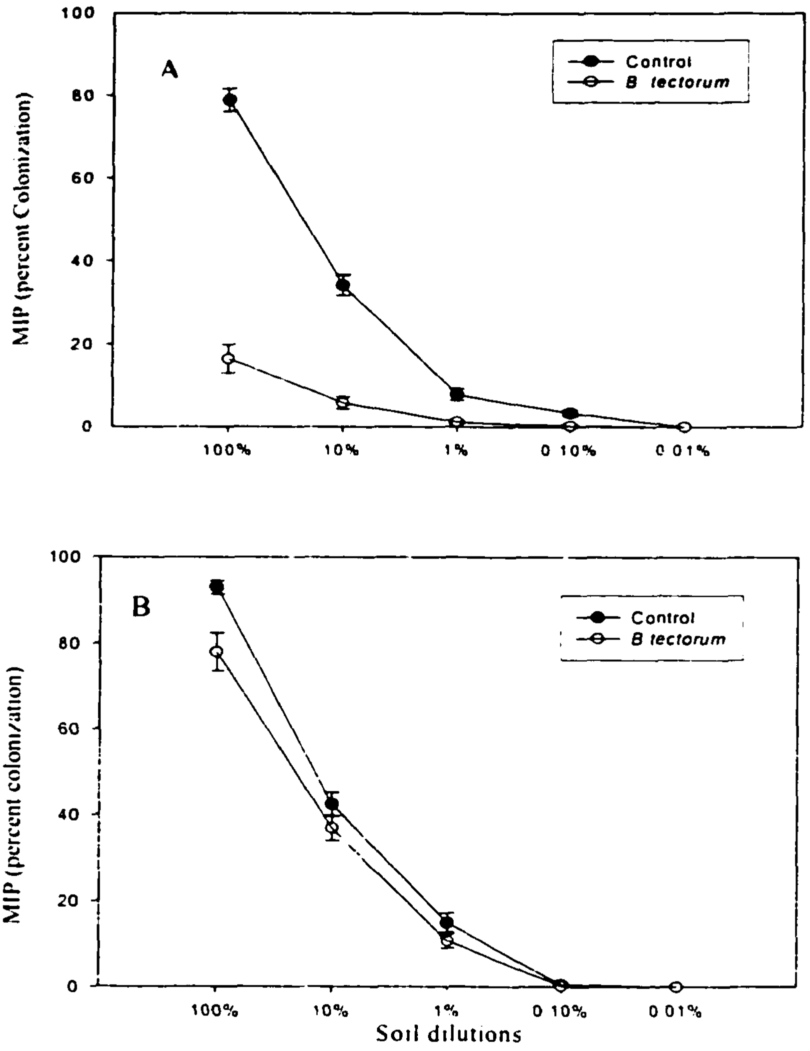

Figure 3.1. Mycorrhizal Inoculum Potential as measured by percent root colonization of bioassay maize plants grown in different soil dilutions collected from patches dominated by *Bromus tectorum* plants and patches without *B. tectorum* plants (Control) in the (A) Central Plains Experimental Range (CPER) and (B) Piceance Basin of northwestern Colorado.

3.1.3. The Spore Counting Method (SC)

Spores abundance of VAM fungi of soil samples collected from the CPER were significantly influenced by *B. tectorum* dominance (Table 3.1). However, difference between the means was very high between the two patches in CPER (one-way

ANOVA: F = 12.64, $p = 0.003$). Spore counts ranged from 24.01 ± 2.38 spores in *B. tectorum* patches to 51.40 ± 7.33 spore gm^{-1} dry soil in control patches. In the Piceance Basin soils, the average spore counts tended to be same in the both soils and there were no significant differences. In *B. tectorum* soils the average SC was100.69 ± 22.1 spores, while SC was102.39 ± 8.72 spores gm^{-1} dry soil in control soils (one-way ANOVA: F = 00, $p = 0.946$) (Table 3.1.). Also, the mean spore counts in Piceance Basin soils exceed the CPER spore counts in both treatments.

3.2. The Relationships between the Study Methods

Figure (3.2.) shows the linear relationship between the $\log_{10}$ of MPN of infective propagules in the inoculum and the percentage colonization of the host plants in *B. tectorum* and control soils collected from the CPER and Piceance Basin. The log of the MPN value was directly proportional to the percentage root colonization (MIP) of the CPER soils (R =0.92, $p = 0.009$). The results for Piceance Basin soil samples were quite similar (R = 0.86 and $p = 0.028$).

The linear relationship between the percentage colonization (MIP) and spore numbers of both *B. tectorum* and control soils in CPER soils was strong and significant (R = 0.815, and $p = 0.048$) (Figure 3.3, A). However, for the Piceance Basin soils, the linear correlation coefficient was less and non-significant (R = 0.516 and $p = 0.295$). (Figure 3.3, B). There was a general trend, but not significant, between the MPN values and spore numbers for both soil sources and in both sites. The linear correlation coefficient between the two study methods for VAM fungi density was (R) = 0.761 and $p = 0.079$ at the CPER site while it was (R) = 0.649 and $p = 0.168$ for soils from the Piceance Basin (Figure 3.4).

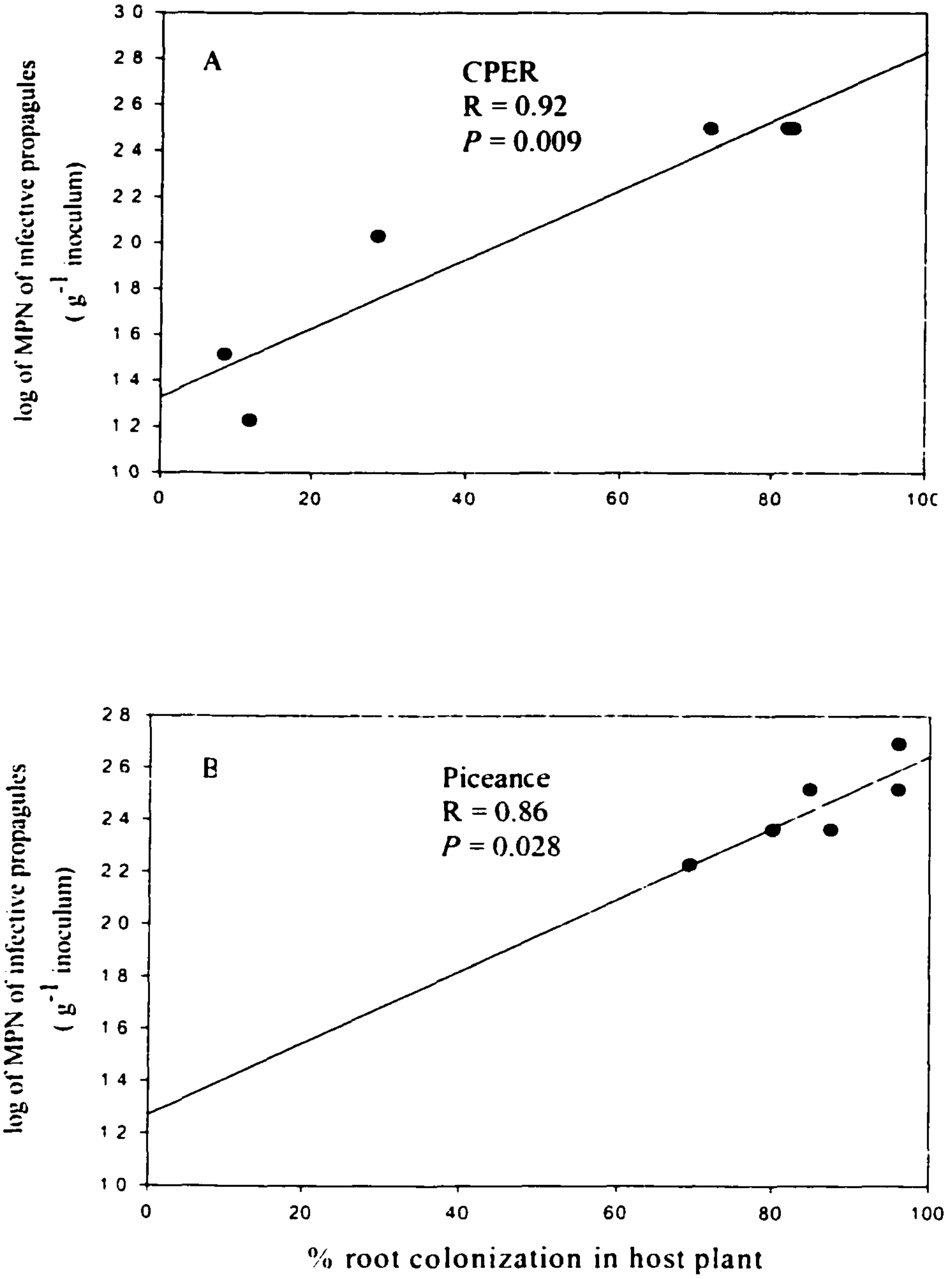

Figure 3.2. The relationship between the MPN of infective propagules in the inoculum (expressed as log MPN) and the percentage infection of both *Bromus tectorum* and control soils in (A): CPER soils, (B): in Piceance Basin soils.

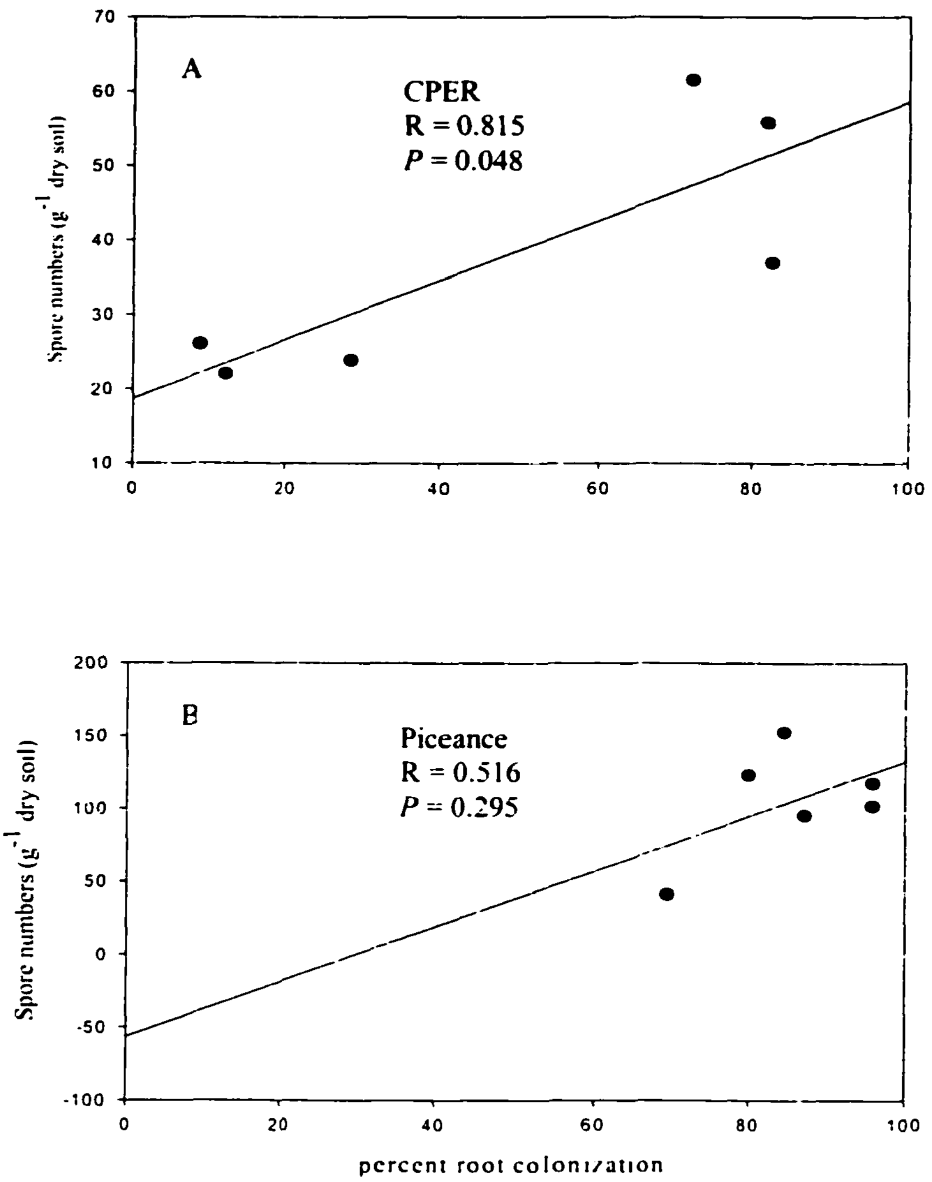

Figure 3.3. The relationship between the percentage colonization (% MIP) and Spore numbers of both *Bromus tectorum* and control soils in (A) CPER soils, (B) Piceance Basin soils.

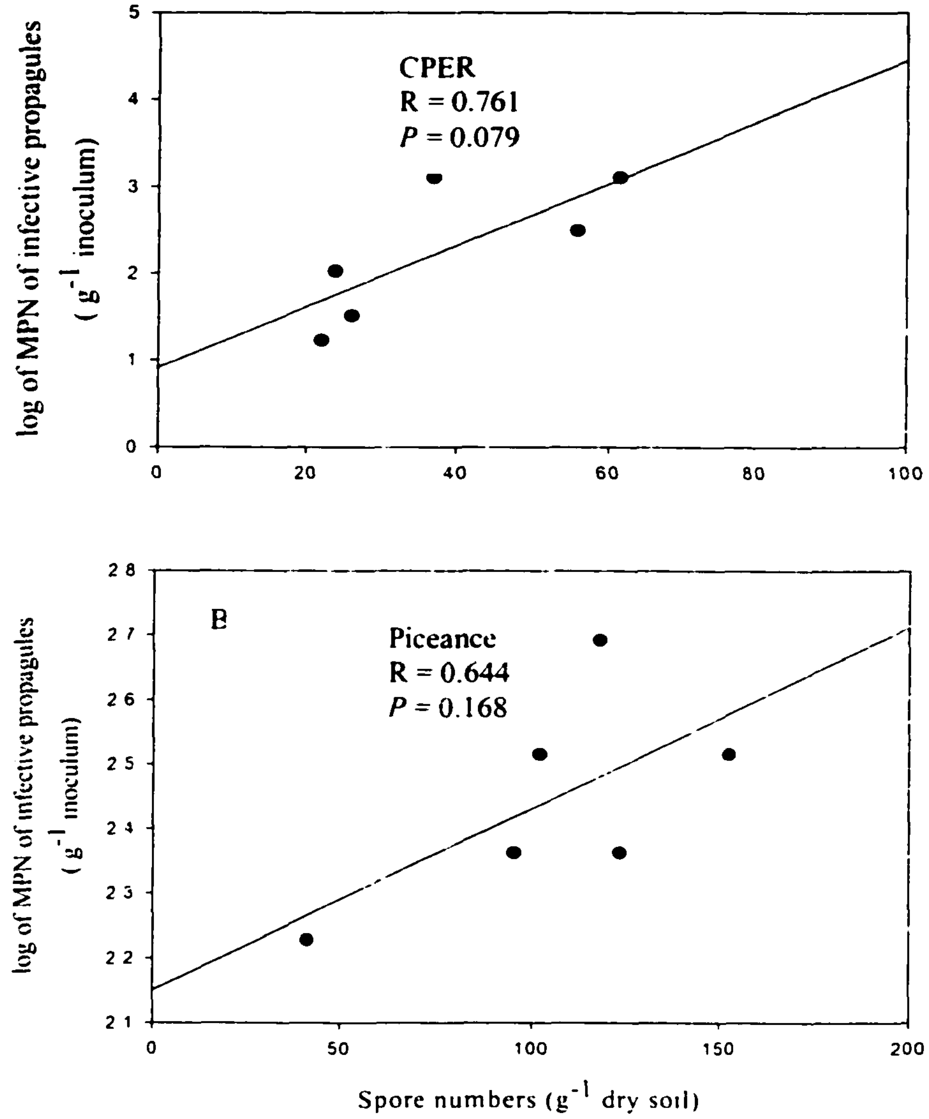

Figure 3.4. The relationship between the MPN of infective propagules in the inoculum (expressed as log MPN) and spore numbers of both *Bromus tectorum* and control soils in (A) CPER soils, (B) Piceance Basin soils.

4. Discussion and Conclusions

4. 1. Effect of *B. tectorum* on VAM Population

Despite the relatively small experimental area and the wide range of variation among plant patches, both the MIP and MPN bioassays and the spore count estimates suggest that either plant species present at a site have a great influence on the occurrence of VAM fungal propagules or the VAM fungi present have a great influence on the plant species present. The results from corn bioassays and VAM fungal spore counts in soils collected from different plant patches, patches dominated by *B. tectorum*, and patches with several plant species but not *B. tectorum*, showed significant differences in VAM fungal population among the two sites in Colorado. Growth-chamber and laboratory studies showed that VAM fungal densities were significantly lower on the patches dominated by *B. tectorum* plants when compared with patches without this exotic weed, especially in CPER. These results indicate that VAM fungi respond differently to the host plant species, and that the different host plant species may play an important role in the maintenance of species composition and diversity in VAM fungal communities.

These data support the hypothesis that VAM fungal population density is significantly reduced in the presence of *B. tectorum*. Also, the three different methods (MIP, MPN, and SC) used to study VAM fungal density indicated the same trends. *B. tectorum* may alter the soil environment when introduced to a site, and the different composition of the VAM fungi beneath *B. tectorum* is the result of induced variation in the soil microenvironments. These results agree with those of Eom (1998). He found in field and greenhouse studies that host plants alter their soil environment and the different composition of VAM fungi under different host plant species respond to this alteration. He suggested that host plant species likely have direct effects on the composition of their associated VAM fungi.

When *B. tectorum* dominates a site, few or no perennial species coexist with it. In such cases, *B. tectorum* is considered a monoculture species. The decrease in VAM fungi population beneath *B. tectorum* may be due to the monoculture effect on VAM fungal density. Johnson et al. (1991) reported that a specific crop monoculture can favor some VAM fungal species over others, and the species will vary in accordance with the host plant. Other research indicates that VAM fungal colonization may be negatively affected by continuous monoculture cropping (Baltruschat and Dehne 1989). Another possible explanation for the low numbers of VAM fungi propagules detected in *B. tectorum* soils is that the growth environment or the host plant employed (corn) is unfavorable for spore germination or mycorrhiza formation by species of VAM fungi adapted to the *B. tectorum*.

The different effects of plant species on VAM fungi in different sites may be due to the fact that each plant species is associated with a different cadre of VAM fungal species, which in turn react differently to other plant species. This assumption also is supported by observation by Strzemska (1975).

Koske and Gemma (1990) reported that there is evidence to suggest that not only do the plants regulate mycorrhizal involvement, but the fungus may also choose or refuse a particular root based on the exudates that a root produces. The attraction and colonization of a mycorrhizal fungus can be based on either a fluctuation in ion transport across the host cortical cell membranes or the recognition of root exudates by the mycorrhizal fungal spore or hyphae (Berbara et al. 1995). In *B. tectorum* a restricted number of VAM fungal species may be associated with this weed and colonize its roots (Chapter 2), while a large number of other fungal species commonly found in sage-grassland are found in more diverse habitats. Those VAM fungal species that cannot form an association with *B. tectorum* roots in the patches dominated by this weed will not have a carbon source, and they would be expected to subsequently disappear or at least decline. Thus, when *B. tectorum* dominates a site or micro-site the VAM fungal density would be expected to be reduced. These results support this conclusion and the observation that a variety of host plants is an important factor that can regulate VAM fungal density.

The reduction of the VAM fungal population with weedy species and the increase of the VAM fungal population with perennial species found in this study agree with Francis and Read (1992) and Wicklow-Howard (1994) who reported a similar relationship between plants and mycorrhizal fungi. They mentioned that many weedy species are either non-mycorrhizal or facultatively mycorrhizal, and, in contrast, VAM fungi colonize mostly perennial species. Hetrick et al. (1988 and 1991) also found that the warm-season grasses with coarse root system are significantly more dependent on mycorrhizae than cool-season, finer-roots species. Since *B. tectorum* is a cool-season grass with a fine root system, one would expect this taxon to be less dependent on mycorrhizae.

The decline of both species richness and abundance of mycorrhizal propagules has been correlated with changes in aboveground species diversity and altered ecosystem functioning (Anderson et al. 1984; Janos 1996). Hetrick et al. (1992) and Wilson and Hartnett (1997) found that plant species in the tallgrass prairie exhibited a wide range of mycorrhizal dependencies, from obligate to facultative mycotrophs. In tallgrass prairie plants growth form and root system morphology is strongly related to the responsiveness of each host species to VAM fungal colonization (Eom 1998).

B. tectorum might increase its competitive advantage and thus, slow the recovery of disturbed soils by affecting the soil microbial community, especially the VAM fungi. Paschke et al. (2000) found that *B. tectorum* was the dominant species at the mid-seral stage of old-field succession in the short grass steppe. The mid-seral stage was characterized by low rates of litter decomposition, high littermass, and very low active fungal biomass in soils relative to the younger (early-seral) old-field. They conclude that these observations suggest that *B. tectorum* might increase its competitive advantage and thus, retard recovery by negatively impacting the soil microbial community and N-cycling processes.

The VAM fungal populations as measured by three different methods in soil from the CPER sites were less than those in soils from the Piceance in both patches with and without *B. tectorum* plants. The different history of the CPER and Piceance sites probably accounts for these differences. The CPER site was cultivated in crops for many years and then abandoned and allowed to remain fallow since 1981. Thompson (1991) found that long periods of fallow land in the eastern grain belt of Australia led to significant decreases in VAM fungal root colonization and the number of spores in subsequent crops. We also know that during crop cultivation both mineral and organic nutrients are often added. These additions also may have adversely impacted on VAM fungal inoculum potential at the CPER site.

Miller and Jackson (1998) mentioned that a large proportion of VAM host plant species in the crop rotation was positively associated with an increase in VAM spore number in the soil, and showed a strong positive correlation with root colonization as well. And they found that if the previous crop was a VAM host, the sampled lettuce crop (their bioassay host species) was more likely to have higher VAM root colonization and higher spore numbers.

The *B. tectorum* patches in CPER had reduced VAM fungal density when compared to comparable patches in the Piceance Basin. With one exception, there were no significant differences between the *B. tectorum* and control soil in terms of their VAM fungi density, when studied by three different methods. A possible explanation is that at the CPER site where the soil samples collected from, few other species occurred with *B. tectorum* plants. On the other hand, I consistently observed that those patches dominated by *B. tectorum* plants in Piceance had perennial species that coexisted with *B. tectorum*.

4. 2. Comparisons between the Three Quantitative Procedures

I compared the three methods commonly used for studying VAM fungal population density. The numbers of infective propagules (MPN) in both sites show a strong correlation with the amount of infection (MIP) in the roots of bioassay plant (R = 0.86 -- 0.92) (Figure 3.2 A and B). For the CPER site, the high associations between spores count (SC) and both MIP and MPN indicate that the most VAM fungi propagules were in the spore form (Figure 3.3. A and 3.4. A). The lack of a strong relationship between MIP and SC and between MPN and SC in Piceance soils, implies that VAM fungi spores are not the only major viable propagules in soil: other propagules such as hyphal fragments and colonized root segments serve as CFUs in Piceance Basin soils (Figuer 3.3.B and 3.4. B).

For both sites, the correlation between MIP and SC is higher than is the correlation between MPN and SC. The strong relationship between MIP and SC in this study finds support in the Furlin and Fortin (1973) data where the percentage infection was directly proportional to the log of the number of spores present.

Each method had some advantages and some disadvantages. There are several clear advantages in using the MPN and MIP techniques over SC. Bioassay methodology results in more uniform recovery of a microbial population across different soil types than spores count (SC) methodology. Another advantage of the bioassay techniques is that, unlike direct spore count, it measures only live and active organisms. Direct counting techniques often are unable to differentiate live from dead organisms. Also, more realistic estimates of the number of infective propagules of VAM fungi in field soils are provided by bioassay techniques when compared to the traditional method of direct spore counting (Porter, 1979).

There is an enormous range of reported VAM fungal spore numbers per gram of soil. With few exceptions, previous research generally reported relatively low numbers of VAM fungal spores/gram soil. Examples included: 6.7-10.4 (Moorman and Reeves 1979), 2-10 (Porter 1979), 0.4 - 25.9 (Allen and Allen 1980), 4.4 - 11.8 (Berliner and Torrey 1989), 1.4 - 8.6 (An and Hendrix 1990), 7-11 (Gange 1993), and 0.1 - 0.25 spores/gm soil (Asbjornsen and Montagnini 1994). However, the method that used here extracted 25- > 200 spores/gm soil. Many of these spores probably were non-viable and were extracted by the flotation method because they contained an air bubble. Even viable spores often float easily because they contain extensive oil droplets. In any case, the methods used here are superior to those previously reported, at least in terms of retrieving VAM fungal spores from soil.

Despite the numerous advantages of using MPN methodology there are a few disadvantages to this method. For example, MPN procedures tend to require more labor and materials than MIP and SC procedures. Also, MPN estimates often have a lower order of precision than do well replicate direct spore counts. The environmental factors, type of inoculum, and manipulations of the inoculum all will affect the results of MPN and MIP techniques (Wilson and Trinick, 1982). Thus, comparisons are not possible between different types of inocula or trials carried out at different times by different people. All relevant comparisons must be made in one experiment (Adelman and Morton, 1986).

Bakarr (1997) found that production of mycorrhizal fragments could pose a problem for MIP or MPN estimates involving bioassays. He mentioned that fragmentation during soil mixing or dilution in MIP and MPN bioassay techniques could decrease infectivity if root fragments are too small.

These present studies suggest that both MIP and MPN measures may not be reliable for land management purposes because of potentially altered colonization of propagules, and the potential lack of correlation with plant performance under field conditions. Therefore, the use of field bioassay to assess MIP or MPN is essential for management because mycorrhiza formation and the effects on a host plant can be difficult to predict under field conditions.

Spores are resilient propagules of VAM fungi and have the potential for prolonged survival in soil. However, there are a number of criticisms of the direct spore counting (SC) method (Porter 1979; Gange 1993). Bakarr (1997) maintained that the enumeration of spores does not indicate the rates at which mycorrhizae will form in the field. Also, this technique may not be appropriate for certain species. For instance, some species only sporulate within roots and so non-spore propagules would be missed (Porter 1979). Moorman and Reeves (1979) also found no relationship between mycorrhizal spore numbers and the colonization of roots; thus, propagules other than spores may be more important in establishing colonization in plant roots.

The relationship between the VAM fungal colonization and the spore count also would depend on spore viability. Sometimes the spore count numbers exceed the expected colonization percentage and at other times they are less. Adelman and Morton (1986) and Allen and Allen (1980) found that spore counts always exceeded MPN estimates, and their results were contrary to those of Porter (1979) and Wilson and Trinick (1982). Miller and Jackson (1998) also found that both VAM colonization and spore number were affected to different degrees by various management practices, and did not always respond similarly. They found spore number also varied between fields, and did not follow the same trends as root colonization.

Many factors affect VAM fungal colonization in host roots. Experimental conditions play an important role in estimating the number of colonizing propagules of VAM fungi by bioassay methods. Allen and Allen (1980) quoted Nicolson (1960) who suggested that the lack of a relationship between the SC and MIP or MPN might be due to three factors that influence mycorrhizal colonization: 1) host plant genotype, 2) edaphic factors, and 3) microbial activity of the soil. The time of harvesting the host plants also is an important factor that affects the bioassay results (Wilson and Trinick 1982). Plant root density also is an important factor in determining the accuracy of the MIP or MPN values. And when the propagules of VAM fungi are either very small or can only grow short distances, root growth will be particularly significant (Wilson and Trinick 1982).

The strong relationship between SC and MIP and MPN are supported by other field and greenhouse observations. Onguene (2000) found that the spore numbers and mycorrhizal colonization in intact and disturbed soil cores were significantly positively correlated and he indicated that mainly spores contributed to the MIP. Graham and Fardelmann (1986) reported a significant linear correlation between number of mycorrhizal fragments per gram of soil and MPN estimates of propagules in the same soil. Also, Fischer et al. (1994) obtained similar results with spores and MPN estimates from both *Allium cepa* and *Psidium guajava* bioassay host plants. Baum and Makeschin (2000) also found similar differences in VAM fungal colonization and spores density between two soils dominated by two different plant clones. However, analysis of spore populations remains one of the few methods to assess the species composition of VAM fungal communities

Discrepancies between spore counts and bioassay estimate (Moorman and Reeves 1979; Porter 1979; Wilson and Trinick 1982; Adelman and Morton 1986) can arise when colonized root fragments act as propagules (Tommerup and Abbott 1981). Adelman and Morton (1986) suggested that bioassay (MPN) estimates only reflect infectious propagules of VAM fungi in the inoculum. This is likely in a bioassay because root fragments are susceptible to soil manipulation, which subsequently affects both their colonization and plant host growth (Bakarr 1997). Bakarr (1997) mentioned that spores are capable of remaining dormant for some time even in the absence of a suitable host, whereas colonized root fragments may rapidly deteriorate. However, Tommerup (1992) found colonized root fragments to be the longer-lasting colonization propagules under her study conditions. Fischer et al. (1994) reported that spores may have been only important colonizing propagules because extracted soils were seeded with bioassay hosts three months after soil was collected from the field. Also, Bakarr (1997) mentioned that results of his MPN and direct bioassay (in the field) suggest that the bioassays are more consistent with each other than they are with indirect measures of single propagule types (spores or root fragments). If the bioassay is begun a short time after collecting soil and with fast growing host plants, the root fragments may remain highly infective. Powell (1976) mentioned that soil has a greater inoculum potential than that suggested by spore counts alone because soil contains roots with viable hyphal fragments.

The several approaches used to assess VAM fungal populations all have some limitations (Dalpé1992). For example, counting spores provides information on only a fraction of the VAM fungi since other mycorrhizal propagules are not accounted for. The MPN and MIP procedures lose information because inoculum potential of the inocula are homogenized and redistributed through the soil (Onguene 2000). The MPN and MIP techniques enumerate many of the propagules that can colonize the test host plant but both techniques fail to detect dormant spores or spores that germinate slowly, and both techniques are sensitive to host species selectivity, in addition to many other problems (Liu and Luo 1994).

The bioassay method provides a more accurate assessment of population (at the two sites studied) than did spore counting method. The bioassay techniques (MIP or MPN) offer alternative but similar methods for comparing population in soils, wherein only the active VAM fungal propagules, under variable soil conditions, are measured (Moorman and Reeves 1979).

I agree with Bakarr (1997) who noted that to obtain a perfect VAM fungal diversity and density assessment technique, minimal soil manipulation after extraction is required or the bioassay should be performed in the field. Janos (1996) also argued that the most ecologically appropriate bioassay is likely to be one conducted in the field and that measures MIP in units of time instead of numbers of propagules or colonization in field collected roots. Bakarr (1997) suggested a technique that involved transplanting host bioassay plants to the field sites and monitoring their growth rates at frequent intervals by non-destructive morphometric measurements. The results obtained from such a field bioassay would indicate within site heterogeneity in both infectivity and effectiveness of VAM fungi, which is appropriate to practical management of disturbed lands. However, this proposal assumes that the *in situ* bioassay plant will show measurable morphometric changes. Many plants do not show significant aboveground responses to colonization by VAM fungi.

Finally, using a soil that is low in mycorrhizal inoculum and is colonized by non-mycorrhizal or facultative mycorrhizal annuals may retare the reclamation of disturbed sites with mycotrophic species. Further studies are necessary to monitor changes in VAM fungal diversity over time and plant community composition, and to assess of the functional significance of landscape level diversity of VAM fungal communities on above-ground plant community composition.

Chapter 4

The Role of Vesicular-Arbuscular Mycorrhizae on Ability of *Bromus tectorum* to Compete with Perennial Native Species

1. Introduction

Vesicular-arbuscular mycorrhizal (VAM) fungi form symbioses in the majority of terrestrial plant families (Trappe 1981). VAM fungi grow around and within root tissues and can influence nutrient uptake, water flow, and other critical parameters of plant health, resulting in enhanced plant survival and growth in resource-limited conditions (Reeves 1985; Smith and Read 1997). Mycorrhizae have the potential to influence plant competition and are important in establishing and maintaining the structure and composition of plant communities (Miller 1987). In several field studies, mycorrhizal colonization has been shown to increase plant growth and survival (Hayman and Mosse 1979; Call and Davies 1988), and many laboratory experiments indicate that a number of plant species benefit from VAM fungi associations in terms of their growth rate (Koucheki and Read 1976; Gange et al. 1990).

Mycorrhizal fungi may play a significant role in determining plant community composition and productivity because of the variation among plant species in their response to mycorrhizal colonization (Allen and Allen 1984; 1986; Benjamin and Allen 1987: Hetrick et al. 1989). The establishment of introduced plants, including invasive species, may be promoted or inhibited depending on the interactions among these plants and resident fungi (Richardson et al. 2000).

The availability of nutrients in soils can vary greatly at spatial scales relevant to individual plants. Under nutrient-limiting conditions, VAM fungi are able to enhance plant nutrient uptake by various mechanisms, thereby over coming plant stress. Plants compete, and under the conditions of limited nutrients, mycorrhizae modify the competitive interactions between plants (Pedersen 1995; Watkison and Freckleton 1997). "Plant nutritive demands vary among plant species. If proper nutrients are available, many plants will grow normally without mycorrhizal fungi. However, without adequate amounts of essential elements the plants will grow poorly or not at all" (Stubblefield et al. 1987). A major contribution of VAM fungi to plants is increased assimilation of relatively immobile soil nutrients such as phosphorus, especially when soils are deficient in these elements (Hayman 1983).

The alien weed cheatgrass, *Bromus tectorum* L., was introduced into the North America continent from the Mediterranean area of Europe, and it is considered a major weed problem in the United State rangelands. *B. tectorum* is adapted to wide range of precipitation, and can colonize a wide range of soil conditions (Billings 1994; Pyke and Novak 1994). In addition to its success as a cropland weed, *B. tectorum* colonizes salt desert shrub communities, sagebrush steppe, and ponderosa pine and Douglas-fir forest (Nesse and Ball 1994). *B. tectorum* is a cool-season (C_3) grass, grows most rapidly in spring and fall when soil temperatures are cooler, and flowers in the late spring and early summer (Harris 1990). Because of its root phenology and rate of root growth *B. tectorum* also is able to exploit soil moisture much more effectively than native bunchgrasses, thereby inhibiting the establishment of native perennial bunchgrasses and impeding successional change (Harris 1977).

Trappe (1981), Pendleton and Smith (1983), and Wicklow-Howard (1994) all report that *B. tectorum* is a facultative host of VAM fungi and may be independent of mycorrhizae. They also note that when *B. tectorum* grows in a nonmycorrhizal plant community, it is nonmycorrhizal, whereas when it grows in the presence of mycorrhizal plants, it is mycorrhizal.

Often alien annuals such as *B. tectorum* comprise the early seral stages of succession. The inhibition of secondary successional change by *B. tectorum* suggests it retains its competitive advantage over native species. Miller and Allen (1992) defined competition between plants as "a depletion of resources by an individual plant to the detriment of a neighbor". *B. tectorum* may derive more benefit from symbiosis with VAM fungi than do the native grasses (Buwalda et al. 1985).

VAM fungi appear to play an important role in succession in rangeland systems that have been radically altered. Following disturbance, the lands are invaded by either nonmycorrhizal or mycorrhizal plants species such as cheatgrass (*B. tectorum*) (Wicklow-Howard 1994). The outcome of competitive interactions between plants can be markedly influenced by VAM fungi (Allen and Allen 1986: Grime et al. 1987). The role of mycorrhizal fungi in both seedling establishment and competitive interactions among the seedlings of native and alien grasses is in need of study. Several studies have been done on the role of VAM fungi in the balance of competition between grass species (Newsham and Watkinson 1998). Species demonstrating less mycorrhizal dependency may show negative responses to mycorrhizal colonization, while more strongly mycorrhizal species show positive responses (Smith and Read 1997).

VAM fungi respond differently to the host species, and the different host plant species may play an important role in the maintenance of species composition and diversity in VAM fungal communities (Eom 1998; van der Heijden et al. 1998). Mycorrhizal dependency in a plant species has been defined as "the degree to which a plant is dependent on the mycorrhizal fungi condition to produce its maximum growth or yield, at a given level of soil fertility" (Gerdemann 1975). It can be calculated by expressing the dry weight of a mycorrhizal plant as a percentage of the dry weight of a nonmycorrhizal plant at a given level of soil fertility (Menge at al. 1982). The dependency of plants on mycorrhizal symbiosis varies, but for plants in undisturbed sites and native ecosystems, the relationship is considered very important (Linderman and Pauliz 1990). Based on the research by Hetrick et al. (1992, 1993), wild plants not only become colonized by VAM fungi to a greater extent than cultivated crops, but they may have a greater dependency on the mycorrhizal symbiosis.

Typically, VAM fungal communities are composed of a complex mix of species, and, importantly, each individual fungal species may have a range of effects on different hosts. Ozinga et al. (1997) found evidence for differences in mycorrhizal affinities for certain hosts, suggesting that host seedlings are differentially colonized by mycorrhizal fungi from different sources, and that there are differential effects on host growth depending on the source of the fungal inoculum. These effects appear to depend upon the VAM species genotypes and community structure, their compatibility with host species, and on the environmental conditions in which the plant is growing.

Since perennials often co-exist with non-mycotrophic annuals on disturbed soils (Allen 1984), understanding of their competitive interactions with and without mycorrhizae would improve our ability to predict the rate of succession after disturbance. In Chapter 2 and Chapter 3, I presented data that clearly show that VAM fungi populations are reduced in soils collected beneath *B. tectorum*. In Chapter 2, I found the roots of *B. tectorum* plants to be colonized by VAM fungi in all plots examined. Based on my findings two important questions arise: 1) do *B. tectorum* plants depend on VAM fungi for their growth and competition with other native species for limiting resources?, and 2) do the symbiotic interactions with VAM fungi help native plant species resist competition from *B. tectorum* plants when they coexist?

The overall objectives of the study in this chapter were to determine if the *B. tectorum* is dependent on VAM fungi for growth and for successful competition with native plant species and to determine the effects of VAM fungi and competition on growth of the two grass species, namely an exotic annual species (*B. tectorum*) and a native bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh.) A. Löve).

P. spicata is a cool season, drought tolerant, long-lived, perennial bunchgrass that is widely distributed and adapted to most sites including thin, non-productive soils. This taxon starts growth in early spring, has a relatively short cycle to maturity, and renews growth in fall (Hirris and Wilson 1970).

I was interested in testing the hypothesis that in interspecific competition between *B. tectorum* plants and a native perennial mycorrhizal species (*P. spicata*), the VAM fungi would more heavily colonize the roots of *P. spicata* than the *B. tectorum* roots. I also wanted to examine the hypothesis that the competitive ability of *B. tectorum* would decrease and that the competitive ability of the native would increase in the presence of VAM fungi. I expected that *P. spicata* would perform comparatively better in the presence of VAM fungi. I wanted to test a third hypothesis that the number of VAM fungal propagules in soil beneath *B. tectorum* plants would be less than the number of VAM fungal propagules in control soil (soil collected from patches free of *B. tectorum* plants). I expected that the number of VAM fungal propagules beneath *B. tectorum* would be less than the number of VAM fungal propagules in control soil. The last hypothesis tested in this study was that *P. spicata* is physiologically capable of maximizing growth in the presence of VAM fungi, in contrast to *B. tectorum* that performs comparatively better in the absence of

VAM fungi. I expected to find that the native species depends on VAM fungi and maximizes growth in the presence of these fungi, whereas the *B. tectorum* would be less dependent on VAM fungi and would maximize growth in the absence of VAM fungi. Since these experiments utilize only one native species (*P. spicata*) and one weedy species (*B. tectorum*), inferences that can be drawn from the results are limited.

2. Materials and Methods

A series of experiments with two species, four levels of competition, and two levels of mycorrhizal fungi were designed. All plants were grown in pots (12 cm x 12 cm) in growth chambers and greenhouse. The two species were *B. tectorum* and *P. spicata*. The *B. tectorum* seeds were collected in summer 1999 from the Central Plains Experimental Range (CPER) (107° 46' W, 40° 49' N) at a short-grass steppe site in northcentral Colorado. The *P. spicata* seeds were obtained from a local seed dealer.

2.1. Experiment 1.

In summer of 1999, the first experiments were established to determine the effect of competition on VAM fungi colonization in roots of *B. tectorum* and *P. spicata*. Soil samples were collected at the CPER site (see Chapter 3). Seeds of the two species were sown in plots (12 cm x 12 cm x 10 cm depth) at four competition levels with 5 replicates, allowing each individual plant 36 cm^2 of surface. One week after germination, the seedlings were thinned to final density (four seedlings per pot). The ratios of *B. tectorum*:*P. spicata* were 4:0, 2:2, and 0:4; the plants were grown for 72 days and harvested. Plants were maintained in a greenhouse with an average temperature of 25° C, and 13 hours light. All nutrients except phosphorus (a modified

10% Hogland's solution) were applied at the rate of 50 ml every two weeks for each plot. Plants were watered every other day with tap water. After 65 days from thinning to final density the plants of each species in each plot were harvested gently using tap water, the root systems were cut from shoots, and the shoots were dried to constant mass at 55° C and weighed. The total root volume of each plant was determined by the water-displacement method. Root systems then were cut into 1-cm segments, and randomly selected segments were fixed in FAA (37% formalin, glacial acetic acid, 95% ethyl alcohol, and water 2:1:10:7 v/v/v/v) for 48 hours. The fixed roots were washed and placed in 7% KOH at 90° C for 60 min; after clearing, the KOH was removed, and samples were washed in water. Samples were then soaked in the acidified water for 10 min, and stained in 0.05% Trypan Blue in lactoglycerin for 10 min at 90° C (Phillips and Hayman 1970). The percentage colonization was calculated as the number of segments with any colonization out of a sample of 100. Only those segments containing mycorrhizal hyphae and either vesicles or arbuscules were counted as colonized.

The data were analyzed using SAS (SAS Institute 1989). One way analysis of variance (ANOVA) was used to test for the effect of competition between *B. tectorum* and *P. spicata* on VAM fungal colonization in the roots, dry shoot biomass, root volumes, and root:shoot ratio of both species. Fisher's least significant difference (LSD) procedure was used to compare the means.

2.2. Experiment 2.

In summer of 2000, soil samples were collected from CPER (see Chapter 3) with a soil core (3.5 cm x 15 cm depth). Soil was sieved to 2 mm, and two experiments were established. The first experiment was a repeat of the 1999 experiment to determine VAM fungi colonization in roots of *B. tectorum* and native species in terms of the effect of competition between the two species; however I used 6 plants rather than 4 plants per pot. Seeds of the two species were sown in 32 pots (4 competition levels with 8 replicates) with a surface area of 144 cm^2, giving each individual plant 24 cm^2 of surface. One week after germination, the seedlings were thinned to final density (six seedlings per pot; *B. tectorum*:*P. spicata* were 6: 0, 3 : 3, and 0: 6); the remaining plants were grown for 72 days and harvested.

The second part of the 2000 experiment was to determine the relative mycorrhizal dependency (RMD) of *B. tectorum* and *P. spicata*. *B. tectorum* and *P. spicata* were grown in soil with and without VAM fungal propagules and in monoculture and in competitive conditions (both species present). The nonmycorrhizal (M-) soil used was sterilized by autoclaving at 121° C for 40 min. on two consecutive days. A portion of unsterilized soil was washed through successive sieves to 106 µm and then through a 45-µm sieve. The filtered water was collected and added to autoclaved soil (5 ml/pot) to re-introduce those native microorganisms other than VAM fungi (Rovira and Bowen 1966) and then incubated at room temperature for 10 days, tested for the presence of VAM fungi, and then used as mycorrhiza-free soil in this experiment.

Seeds of the two species were sown in 30 pots (4 competition levels and 2 levels of mycorrhizal status (M+) and (M-) soils with 5 replicates) with a surface area of 144 cm^2, giving each individual plant 24 cm^2 of surface. One week after germination, the seedlings were thinned to a final density (six seedlings per pot; *B. tectorum*:*P. spicata* were 6:0, 3:3, and 0:6); the remaining plants were grown for 72 days until harvested. Plants were maintained in a greenhouse with an average temperature of 25° C, and 13 hours light. All nutrients except phosphorus (a modified 10% Hogland's solution) were applied at the rate of 50 ml every two weeks for each plot. Plants were watered every other day with tap water. After 65 days from thinning to final density, the plants for each species in each plot were harvested gently using tap water, and the shoot height and root length, were taken. Then roots were cut from shoots, dried separately to constant mass at 55° C and weighed.

In the first part of the 2000 experiment, a complete random design (CRD) was used, with 8 plot replicates (12 cm x 12 cm x 10 cm depths). Treatments were assigned as four competition levels. One-way analysis of variance (ANOVA) was used for each treatment to evaluate the effects of presence of VAM fungi on VAM fungal colonization and growth characteristics (shoot biomass, root biomass, and root:shoot ratio).

In the relative mycorrhizal dependency (RMD) experiment, a complete random design with five plot replicates (12 cm x 12 cm x 10 cm depths) was used to determine if *B. tectorum* and *P. spicata* were dependent on VAM fungi for their growth. Also, the effects of VAM fungi on plant growth characteristics of both species were noted. One-way ANOVA also was used to evaluate the *B. tectorum* and *P. spicata* dependency on VAM fungi (SAS Institute, 1986). Fisher's least significant difference (LSD) procedure was used to compare the means.

The relative mycorrhizal dependency (RMD) was calculated by expressing the difference between the mean total dry weight of mycorrhizal plants (M+) and the mean total dry weight of nonmycorrhizal plants (M-) as a percentage of the mean dry weight of mycorrhizal plants in a given soil (Plenchette et al. 1983).

The equation for this calculation is:

$$\text{RMD} = \frac{(\text{Dry wt. M+ plant}) - (\text{Dry wt. M- plant})}{(\text{Dry wt. M+ plant})} \times 100$$

Plants with a RMD of 100% are considered to be 100% mycorrhizal dependent. Such plants are unable to grow in the absence of mycorrhizal fungi. A RMD value of 0% occur when mycorrhizal plants grow as well as nonmycorrhizal plants, i.e., there is no significant difference in their dry weight.

In addition to determining the relationship between the VAM fungi colonization and the competition between the two plant species, the VAM fungi population density in the two soil sources (soil collected from patches dominated by *B. tectorum* plants and from patches free of *B. tectorum*, control soil) were tested in this experiment using *B. tectorum* and *P spicata* as hosts for VAM fungi.

3. Results

3.1. VAM Fungal Root Colonization and Competition

Table 4.1 shows the mean mycorrhizal colonization of species, *B. tectorum* and *P. spicata* in monoculture and in competition plots for 1999 and 2000 experiments. Colonization of *B. tectorum* roots from mixed plots was significantly greater than *B. tectorum* roots from monoculture plots in both years (1999 and 2000). In 1999 and in 2000, colonization of the *B. tectorum* roots was 87.00 % and 81.88% from mixed plots, and 61.00 % and 71.45 % from monoculture plots, respectively. *B. tectorum* plants increased mycorrhizal colonization in their roots when in competition with *P. spicata*, and colonization in *P. spicata* roots was significantly decreased when in competition with *B. tectorum* in both two years. The colonization percentages of *P.*

spicata roots in 1999 and 2000 were 92.50 % and 86.21 % in monoculture and 74.50 % and 69.67 % when in competition with *B.*, respectively (Table 4.1).

Table 4.1. Mean of mycorrhizal colonization in roots of *Bromus tectorum* and *Pseudoroegneria spicata* in monoculture and mixed conditions for two years (1999 and 2000)

Treatment		% Colonization 1999	% Colonization 2000
B. tectorum	*Monoculture*	61.00^{C*}	71.45^{b}
	Mixed	87.00^{AB}	81.88^{a}
P. spicata	*Monoculture*	92.50^{A}	86.21^{a}
	Mixed	74.50^{BC}	69.67^{ab}
	F	9.58	6.27
	P	< 0.0001	= .008
	LSD	13.58	9.86

* means with same letters in a column represent non-significant differences at $p > 0.05$.

3.2. Plant Growth and Competition

Table (4.2) shows the effect of competition on root volume, shoot biomass and root:shoot ratio for both plant species in two years. In the 1999 experiment, the root volume of *B. tectorum* plants increased significantly when in competition with *P. spicata* plants, whereas the *P. spicata* root volumes were not affected. The root volume for *P. spicata* in both mixed and monoculture plots and the root volume for *B. tectorum* in monoculture plots were similar in 1999. But in the 2000 experiment, there were no significant differences between the root volumes in either the mixed or monoculture plots in both species. On the other hand, there were significant differences between the root volumes of *B. tectorum* and *P. spicata* plants. *B. tectorum* root volume was significantly greater than *P. spicata* root volume in both years.

B. tectorum dry shoot biomass increased significantly when in competition with *P. spicata* in 1999 but not in 2000. *P. spicata* did not show any significant changes in dry shoot biomass between mixed and monoculture plots in either year (Table 4.2). *B. tectorum* plants had higher root:shoot ratios than *P. spicata* plants in 2000 but not in 1999 (Table 4.2).

Table. 4.2. Means of root volume (ml), shoot biomass (g), and root:shoot ratio of *Bromus tectorum* and *Pseudoroegneria spicata* in monoculture and mixed conditions for two years (1999 and 2000)

Treatment	1999			2000		
	Root volume mi / plant	Shoot mass gm / plant	Root: Shoot ratio	Root volume ml / plant	Shoot mass gm / plant	Root: Shoot ratio
B. tectorum						
Monoculture	0.516^{B*}	0.013^{B}	43.17^{A}	0.917^{A}	0.040^{A}	22.97^{AB}
Mixed	2.025^{A}	0.069^{A}	30.31^{A}	1.125^{A}	0.037^{AB}	31.00^{A}
P. spicata						
Monoculture	0.475^{B}	0.015^{B}	37.16^{A}	0.250^{B}	0.025^{BC}	10.12^{C}
Mixed	0.415^{B}	0.010^{B}	46.59^{A}	0.292^{B}	0.020^{C}	16.08^{BC}
F	58.32	34.12	1.29	7.55	4.94	7.43
P	<.0001	< .0001	= 0.31	= 004	=.018	=.005
LSD	0.306	0.014	18.89	0.495	0.013	10.17

* means with same letters in a lumn represent non-significant differences at $p > 0.05$.

3.3. VAM Fungal Density in Different Soil Sources

Analysis of variance of colonization values from both bioassay host species indicated that infective propagules of VAM fungi are more numerous in the control soils than in soils from *B. tectorum* (Table 4.3). The VAM fungal population in the control soil was significantly greater ($p = 0.01$) than in *B. tectorum* soils when assayed with *B. tectorum* plants. *P. spicata*, on the other hand, showed a general but non-significant trend of a reduction in VAM propagules in *B. tectorum* soils (Table 4.3).

Table 4.3. Mean of mycorrhizal colonization in roots of *Bromus tectorum* and *Pseudoroegneria spicata* plants grown in two different soil sources- soil with *B. tectorum* and soil without *B. tectorum* (control soil)

	Host plant Percent of colonization	
Soil source	*B. tectorum*	*P. spicata*
B. tectorum soil	59.73^{B*}	76.21^{A}
Control soil	71.45^{A}	86.21^{A}
F	12.23	2.81
P	= 0.013	= 0.145
LSD	8.21	14.60

* means with same letters in a column represent non-significant differences at $p > 0.05$.

3.4. Relative Mycorrhizal Dependency (RMD)

Figures 4.1, and 4.2 show that the presence of VAM fungi in soil usually resulted in increased of growth of both *B. tectorum* and *P. spicata* when they were grown in monoculture plots. In the dependency experiment, *B. tectorum* mycorrhizal plants had significantly greater dry shoot biomass ($p < 0.001$), dry root biomass ($p < 0.001$), and total plant biomass ($p < 0.001$) than did nonmycorrhizal plants. Despite the increased *B. tectorum* shoot biomass in mycorrhizal soils, there were no significant differences between mycorrhizal and nonmycorrhizal *B. tectorum* plants in height ($p = 0.057$). *P. spicata* plants responded significantly to the presence of VAM fungi by increasing dry shoot biomass, dry root biomass, and plant height compared to nonmycorrhizal plants (Figure 4.1 and 4.2).

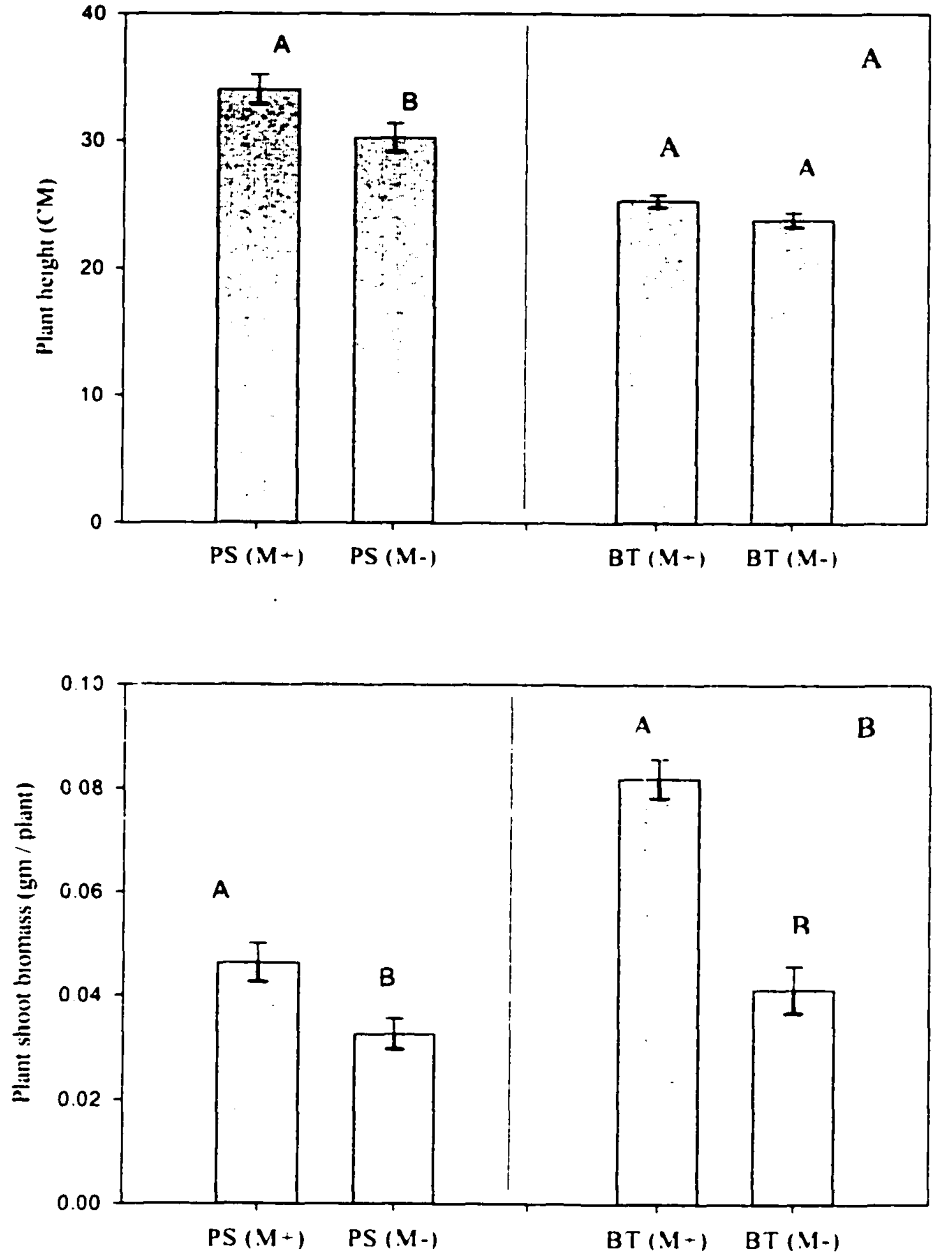

Figure 4.1. Means of (A) plant height and (B) shoot biomass for mycorrhizal *Pseudoroegneria spicata* plants (PS, M+), nonmycorrhizal *P. spicata* plants (PS, M-), mycorrhizal *Bromus tectorum* plants (BT, M+), and nonmycorrhizal *B. tectorum* plants (BT, M-) grown in the growth chamber for 72 days. Each bar represents the mean of five replicates ± SE. Means within a species with the same letters represent non-significant differences at $p > 0.05$.

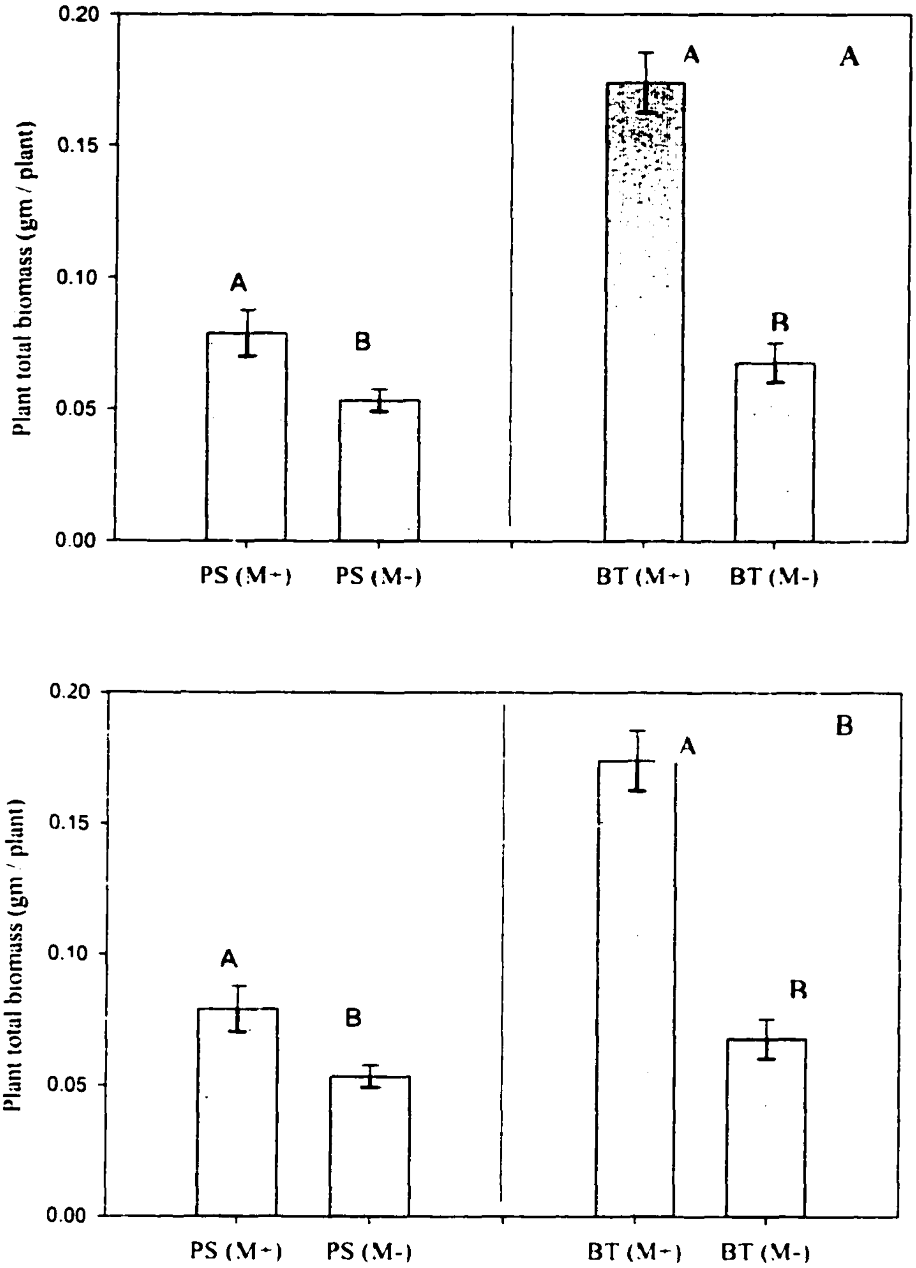

Figure 4.2. Means of (A) root biomass and (B) total biomass for mycorrhizal *Pseudoroegneria spicata* plants (PS, M+), nonmycorrhizal *P. spicata* plants (PS, M-), mycorrhizal *Bromus tectorum* plants (BT, M+), and nonmycorrhizal *B. tectorum* plants (BT, M-) grown in the growth chamber for 72 days. Each bar represents the mean of five replicates ± SE. Means within a species with the same letters represent non-significant differences at $p > 0.05$.

Based on relative mycorrhizal dependency (RMD), calculated from dry shoot biomass, dry root biomass, and total biomass, both species are dependent on VAM fungi (Figure 4.3) for growth. *B. tectorum* had greater RMD values than *P. spicata* in all situations. The RMD values of *B. tectorum* were 49.8%, 71.2%, and 61.1% for shoot biomass, root biomass, and total biomass on monoculture plots, respectively, whereas RMD value was 43%, 54.7%, and 47.5 % when in competition. The RMD values of *P. spicata* were 29.5%, 35.4%, and 31.9% in shoot biomass, root biomass, and total biomass in monoculture plots, respectively, and 17.5%, 0.0%, and 11.5% when in competition with *B. tectorum*.

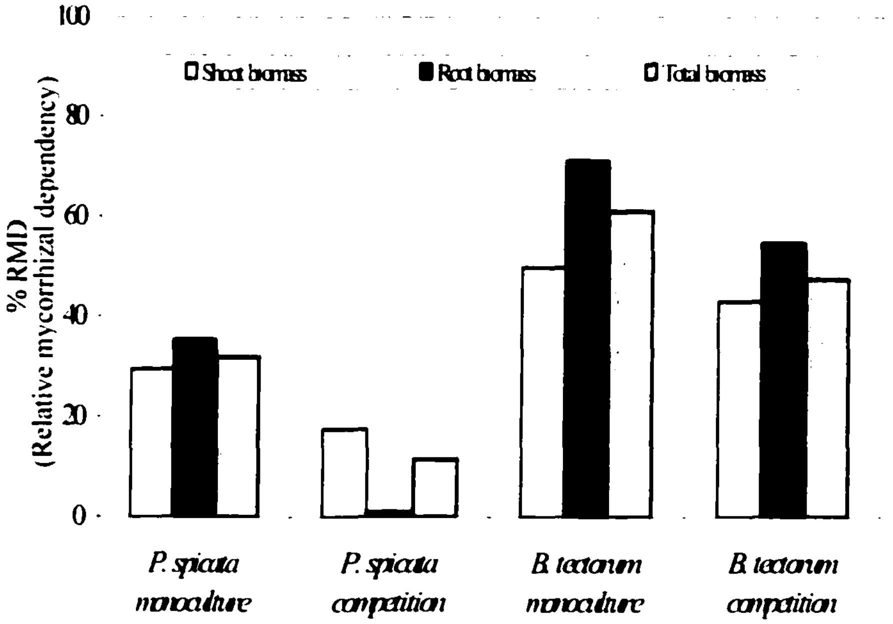

Figure 4.3. Relative mycorrhizal dependency (RMD), calculated from dry shoot biomass, dry root biomass, and total biomass, for *Bromus tectorum* and *Pseudoroegneria spicata* grown in monoculture and in mixed pots.

The presence VAM fungi in the soil increased both the shoot and root biomass of both species. Competition significantly reduced RMD. Both species increased RMD values when grown in monoculture when compared to competitive growth conditions (Figure 4.2).

4. Discussion

I examined the hypothesis that in interspecific competition between *B. tectorum* (an invasive annual C_3 grass) and *P. spicata* (native perennial C_3 grass); the roots of *P. spicata* would be more highly colonized than *B. tectorum* roots. The results from two experiments did not support this hypothesis. Root of the *P. spicata* plants had lower colonization when in competition with *B. tectorum*. However, roots of *B. tectorum* were more highly colonized by VAM fungi than roots of *P spicata* in mixed pots. These observations support the conclusion that *B. tectorum* decreases VAM fungal colonization in other plant species roots, including the native cool season *P. spicata*, when they compete with each other (Table 4.1). *B. tectorum* plants may use this strategy as a competitive tool in order to more effectively sequester limited resources, nutrients, and/or water in the soil while reducing the VA mycorrhizal advantages for other competitor species. These results agree with Ocampo et al. (1980), who found that certain host plants could reduce colonization levels in other plants in the same spot.

Also, I examined the hypothesis that the competitive ability of *B. tectorum* would decrease and the competitive ability of *P. spicata* would increase in the presence of VAM fungi. I found that *P. spicata* did not perform comparatively better in the presence of VAM fungi when in competition with *B. tectorum* (Table 4.2). Thus, these results do not support this hypothesis. In both 1999 and 2000, competition between the two species led to enhanced colonization in *B. tectorum* (Table 4.1). Colonization enhanced the growth of both root and shoot yield in *B. tectorum* and slightly decreases the growth of *P. spicata* in 1999. There were no significant changes between the mixed and monoculture pots in both species, but there were slight changes in the root and shoot growth when they were in competition in 2000. The different number of plants per pot in 1999 and 2000 also affected root colonization by VAM fungi and root and shoot growth. Roots colonization of *B. tectorum* in 2000 was higher than in 1999 in monoculture pots (Table 4.1), perhaps because of the increased number of plants per pot in 2000. Koide (1991) found no positive correlation between mycorrhizal colonization and seedling performance, and he suggested that this might be expected if the benefits from mycorrhizae are lower than the costs for the relationship.

In conditions where relatively few species benefit more from VAM fungi association than co-occurring plant species, the presence of VAM fungi may increase the dominance of these species (Hetrick et al. 1989; Hartnett et al. 1993). Therefore, this may bring about a reduction in plant species diversity. One possible cause of the lack of response to mycorrhizal colonization in *P. spicata* grown in the presence of *B. tectorum* may to due an allopathic chemical effect from *B. tectorum* plants on VAM fungi (Rice 1986; Rose et al. 1983; Pellissier and Souto 1999). The second possible reason for the decrease in VAM fungal colonization in roots of *P. spicata* may be the large root system of *B. tectorum*. However, this reduction in colonization did not have significant effects on the dry weight of *P. spicata* plants (dry shoot and root biomass), whereas increasing mycorrhizal colonization in *B. tectorum* had positive effects in 1999.

The hypothesis that VAM fungal propagules in soil beneath *B. tectorum* plants would be lower than in control soils was tested. Using both *P. spicata* and *B. tectorum* plants as bioassay hosts supports this hypothesis. I found that soil beneath *B. tectorum* had lower VAM fungal density values than VAM fungal density in control soil. The roots of both hosts were less colonized when grown in *B. tectorum* soil than when grown in soil collected from patches free of *B tectorum*. This result agrees with that found in Chapter 3 of this dissertation.

Results gained from the mycorrhizal dependency experiment do not agree completely with the hypothesis that the native species (*P. spicata*) is capable of maximizing growth in the presence of VAM fungi and that the exotic, weedy species (*B. tectorum*) would perform comparatively better in the absence of VAM fungi. Shoot and root biomass of both of *P. spicata* and *B. tectorum* were increased by the presence of VAM fungi (Figures 4.1 and 4.2). When the two species compete in the presence of VAM fungi, roots of *P. spicata* were not affected while the shoots of both species and roots of *B. tectorum* increased (Figure 4.3). Both species clearly benefit from the relation with VAM fungi, and are dependent on that relationship for optimal growth. This conclusion is supported in the dependency experiment (Figure 4.3), where *B. tectorum* and *P. spicata* grew larger in the presence of mycorrhizal fungi. The high RMD for *B. tectorum* might be one of many factors that help this species compete with other species and survive.

In this study, there were no correlations between the level of colonization by VAM fungi and shoot and/or root biomass. One might assume that more colonization by VAM fungi could lead to greater water and nutrients uptake and greater competitive ability for *B. tectorum* plants up to a threshold level. Root colonization above an optimal level may not contribute to dry weight increase. Graham et al.

(1982) found an erratic relationship between the plant growth response and root colonization. Hetrick et al. (1990) reached similar conclusions in their research. They found that root colonization above 20% did not benefit C_4 plants they studied; in these warm-season grasses, root colonization above 20% appears to be superfluous, but not harmful. Since colonization typically exceeded 20% in both *B. tectorum* and *P. spicata*, that could explain why there was no correlation between dry biomass and root colonization.

Most reports note an increased growth response by plants to mycorrhizal colonization. Hayman (1983) attributed increased plant growth to improved nutrient status, especially when elements are limiting. The few reports of growth depressions arising from VAM colonization are attributed to host-fungus competition for carbohydrates (Buwalda and Goh 1982). Some researchers (Nemec 1987) found that plants that were less dependent on VAM tended to have higher root:shoot ratios, but my work and the work of Saif (1987) do not support the premise that dependency on and the benefits of VAM are strongly related to an increase in root:shoot ratios. *B. tectorum* has a higher RMD than *P. spicata*, and *B. tectorum* has a higher root:shoot ratio, in 2000 than *P. spicata*.

The relationship between the responsiveness of a host species to VAM fungal colonization is strongly affected by plant growth form and root system morphology (Eom 1998). If a plant is able to rapidly colonize open space following disturbance, then a competitive advantage might be realized (Eissenstat and Caldwell 1987). *B. tectorum* appears to have the ability to maintain stable populations in disturbed sites for long periods and can retard the succession of native spices. Goodwin (1992) reported that *B. tectorum* may benefit from mycorrhizal colonization immediately after germination by sustaining its root growth in lower, warmer soil horizons during winter, when other native species are dormant, and that the rapid rate of growth of *B. tectorum* would be a strong path for water and nutrients uptake by VAM fungi.

The outcome of the interaction between two species grown together with or without the VAM inoculum may be quite different (Francis and Read 1992). Fitter (1977) found that the *Lolium perenne* yield was reduced by 46% when it was grown with *Holcus lanatus* in the presence of VAM fungi relative to that found when the two species were grown together in the absence of VAM fungi. Hetrick et al. (1989) examined two grass species, *Andropogon gerardii* and *Koeleria pyramidata*, from the tallgrass prairie, and found that in sterile soil (M-), *K. pyramidata* was not influenced by the presence of *A. gerardii*, but in presence of VAM fungi inoculum *A. gerardii* led to a yield reduction in *K. pyramidata* of up to 91% Also, Hall (1978) found that VAM stimulated growth in clover (*Trifolium repens*) to a much greater extent under competitive conditions than when grown alone. Thus, a competitive relationship may produce a situation in which mycorrhizal colonization is detrimental to one species and helpful for another species.

In natural vegetation systems, the temporal separation of vegetative activities might enable the early-season; C_3 species *B. tectorum* to avoid competition of late season C_4 species. Also, the fine fibrous root system of *B. tectorum* might capture nutrients with sufficient effectiveness through most of the life of the host to void any advantageous effects of VAM infection.

In this presence study both species are cool season grasses. Germination rate studies of *P. spicata* and *B. tectorum* indicate that both germinate rapidly, especially at colder temperatures, and early season root growth in both is significantly greater than that found in warm season grasses (Harris and Goebel 1976). Harris and Wilson (1970) found that *B. tectorum* roots can grow at colder temperatures than *P. spicata*, and, when they reach a thermally protected depth, they can continue to grow throughout the winter. *B. tectorum* roots may use all available soil moisture in the spring before the roots of *P. spicata* have begun to grow again (Harris 1967; Harris and Goebel 1976; Harris 1977). Arredondo and Johnson (1998) reported that root development in *P. spicata* is slower than in a *B. tectorum*.

My results disagree with those of Pendleton et al. (1999). They reported that the addition of mycorrhizal fungi significantly decreased the ability of *B. tectorum* to compete with blackbrush (*Coleogyne ramosissima*) seedlings when grown at low soil nutrient levels. But their results were similar to what I found in terms of effects of *B. tectorum* on blackbrush growth in the presence of VAM fungi, especially in fertile soil. They attributed the observed reduction of blackbrush shoot growth in the presence of both mycorrhizae and *B. tectorum* to biomass allocation patterns. Competition resulted in an altered allocation pattern for the shrub, but not the grass (*B. tectorum*). They found that the root:shoot ratio of mycorrhizal plants was reduced in the absence of competition with blackbrush plants. In contrast, they found that the root:shoot ratios for the competing *B. tectorum* plants declined with mycorrhizal fungi.

My results disagree with Gooodwin (1992) who reported that *B. tectorum*'s mycorrhizal dependence is lower than that of a native perennial. He also mentioned that *B. tectorum* did not benefit from mycorrhizal colonization except when the relative density of mycorrhizal competitors was high. Also, my results disagree with what Schwab and Loomis (1987) and Benjamin and Allen (1987). They found that VAM fungi in monoculture inhibited *B. tectorum* growth. I found that VAM fungi enhanced shoot and root growth of *B. tectorum* in both treatments, mixed and monoculture pots. On the other hand, Schwab and Loomis (1987) found that VAM fungi enhanced *B. tectorum* by increased P uptake when it is in competition with *Agropyron spicatum* (old name for *P. spicata*), and *B. tectorum* suppressed growth of *P. spicata* when the two grasses were in competition.

The synergistic effects of nutrient and water uptake are observable when mycorrhizal plant species are placed in competition with non-mycorrhizal or with less mycorrhizal dependent species. Work on *Andropogon gerardii* by Hetrick et al (1994) showed that this species couldn't effectively compete with the cool-season grass *Koeleria pyramidata* in the absence of mycorrhizae. However, if colonized by mycorrhizal fungi, *A. gerardii* is able to outcompete *K. pyramidata*. Therefore, the added benefits of mycorrhizae are sufficient to alter the balance of fitness between these two competing prairie species. Research also has shown that although non-natives may be the first to colonize disturbed areas, succession by mycorrhizal native species can eventually replace the invasive plants (Reeves et al. 1979).

Different fungal species may respond differently to the host plants (Eom 1998). Hetrick and Bloom (1986) observed that spore production of *Glomus fasciculatum* was significantly influenced by some host plant species, whereas spore production of *G. macrocarpum* and *G. mosseae* was not. The host species differ significantly in their dependency on VAM fungal symbiosis (Hetrick et al. 1991; Wilson and Hartnett 1997). The responses of host plant species to VAM fungal colonization ranges from beneficial to harmful (Carling and Brown 1980), and these different responses between host plant species and VAM fungal species could be a result of preferences or ecological specificity between symbionts (Smith and Read 1997; Sanders and fitter 1992).

Many researchers generally assume that VAM fungi do not show host specificity in natural communities because VAM fungi colonize most plant species and because multiple species of VAM fungi can colonize a single plant species (John and Coleman 1983; Sanders and Fitter 1992; Smith and Read 1997). Results from my work show that *B. tectorum* plants depend on VAM fungi as measured by RMD and show an increase in VAM fungal colonization when in competition. That suggests that *B. tectorum* uses the VAM fungi to improve competition with native species by increasing VAM fungal colonization on *B. tectorum* roots and decreasing VAM fungal colonization on the *P. spicata* roots (Table 4.1). *B. tectorum* biomass also increased significantly in mycorrhizal soils (Figures 4.1, 4.2 and 4.3). These results indicate that different host species may influence VAM fungal density. *B. tectorum* may alter the soil environment when it becomes established at a new site, and the population density of VAM fungi beneath *B. tectorum* is reduced as the result of such changes in the microenvironments.

In field and greenhouse studies, Eom (1998) found that host plants altered their soil environment and changed the composition of VAM fungi under different host plant species. He suggested that host plant species likely have direct effects on the composition of their associated VAM fungi. Wilson and Hartnett (1998) found a significant positive correlation between root colonization levels and mycorrhizal dependence of tallgrass prairie plant species. They also measured the ratio of the biomass increase in mycorrhizal plants as compared to nonmycorrhizal plants. They found low spore abundance of VAM fungal communities under *Poa pratensis* L. host plants from both the field and the greenhouse. They suggested that low spore abundance could be the result of low mycorrhizal dependency of the host species and that mycorrhizal dependency or colonization rate are influencing factors in fungal sporulation.

B. tectorum may prefer to form relationships with only certain VAM fungi species during less competitive conditions (i.e., when sufficient nutrients, water, space, etc. are available). Thus, the density and possibly the diversity of VAM fungi decrease in presence of *B. tectorum*. On the other hand, *B. tectorum* plants in unfavorable conditions may increase the VAM fungi species that colonize their roots. I found that *B. tectorum* plants increased the percentage of VAM colonized roots when they compete with native species (Table 4.1). These data agree with Eom (1998), who reported that a host plant species might favor the colonization of certain species of VAM fungal species under certain conditions, and that host plant species are not randomly colonized by all available fungal species. He also reported that the benefit of VAM fungi for a given host plant may vary among different VAM fungal species.

Many researchers assume that VAM fungi are generalists, ubiquitous, and randomly distributed in herbaceous communities, and show low host specificity (Eom 1998). However, Johnson et al. (1991, 1992) reported that in successional grasslands in Minnesota, VAM fungal species composition changed during succession and was highly controlled by the host plant species. In addition, Eom (1998) and Bever et al. (1996) showed that sporulation of VAM fungal species depends on host plant species. Thus, maybe there is some degree of host specificity between VAM fungi and plant species. Results of these present studies suggest that *B. tectorum* maybe a host for specific VAM fungal species that are closely associated with this taxon. Other VAM fungal species may disappear when *B. tectorum* plants are present. Thus, ecological specificity could play an important role, since different VAM fungi colonize only certain plants or determine specific plant responses (van der Heijden et al. 1998; Zhu 2000).

RMD showed that the belowground systems of *B. tectorum* show a greater response to VAM fungi than the aboveground system (Figure 4.3) when *B. tectorum* is in competition with *P. spicata*. However, the aboveground system of the perennial species, *P. spicata*, showed a greater response to VAM fungi than did the belowground system. In this study, *P. spicata* had a 0% mycorrhizal dependency in its root systems when in competition with *B. tectorum*. In grasslands as much as 70% of the biomass is below-ground (Clark 1975). One, therefore, would expect that most of the effects of treatments (fertilizers, mycorrhizal fungi, etc.) would be manifested in the belowground portion of the plants and frequently missed by the scientist whose research focused on the aboveground portion of the community.

5. Conclusion

The invasion of *B. tectorum* into native plant communities may depend on, or is at least greatly enhanced by, the establishment of the mutualism between its roots and VAM fungi. *B. tectorum* responds positively to VAM fungi colonization. Since VAM fungi are important for growth and survival of *B. tectorum*, many questions on the role of VAM fungi and *B. tectorum* establishment remain unknown and should be studied. For example, which of the VAM fungal genera and species are restricted to *B. tectorum* as a host species; how are the mycorrhizal populations structured in space and time; does the presence of *B. tectorum* favor a single VAM fungal species; how might the mycorrhizal community be impacted by non-sustainable management practice, and how might this, in turn, influence *B. tectorum* survival and growth?

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