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Mycorrhizae In Sagebrush-Steppe Community Restoration: Mycorrhizal Dependency Of Invasive And Native Grasses With Intraspecific And Interspecific Competition

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MYCORRHIZAE IN SAGEBRUSH-STEPPE COMMUNITY RESTORATION: MYCORRHIZAL DEPENDENCY OF INVASIVE AND NATIVE GRASSES WITH INTRASPECIFIC AND INTERSPECIFIC COMPETITION

by

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ABSTRACT

Mycorrhizal Inoculum as a Restoration Tool in the Great Basin

by

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Mycorrhizae have been used in restoration for decades. However, studies assessing the use of mycorrhizae in *Bromus tectorum*-invaded areas of the Great Basin are limited. Two greenhouse pot experiments were conducted to assess the role of mycorrhizae in sagebrush restoration.

The first objective (Chapter 2) was to determine the response of *Pseudoroegneria spicatum*, *Elymus elymoides*, and *B. tectorum* to mycorrhizal symbiosis by altering phosphorus, density, species, presence of mycorrhizae and water levels in a 5 factor design. To assess the mycorrhizal response, a variety of morphological and physiological traits were measured, such as tissue P concentration, specific root length, specific leaf area, carbon isotope discrimination, etc. The effects of the different treatment combinations were analyzed using ANOVA.

The second objective (Chapter 3) was to determine the role of different inocula in competition between the three grasses. Species, density, and inoculum type were altered in a 3-factor design. Inoculum was cultured on *Allium* plants. The effect of locally

cultured inoculum on the species was compared to the effect of commercial inoculum. The response of each species to mycorrhizae with different species compositions and densities was assessed. Morphological measurements were used to determine each species response to the different factor combinations. The effects of the different treatment combinations were analyzed using ANOVA. This research provides land managers with information regarding the efficacy of using local versus commercial inocula and whether they should use mycorrhizae in restoring their systems.

(165 pages)

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Dara S. Scherpenisse

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CHAPTER 1

RESTORING GREAT BASIN PLANT AND ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITIES

Dynamics of sagebrush communities have been drastically altered by the introduction of the annual grass *Bromus tectorum*. Among other effects, *B. tectorum* has increased fine fuel loads and fire frequency in a system that is not adapted to short fire intervals. As a result, *B. tectorum* has increased while native species have decreased (Stewart & Hull, 1949; Wright, 1985; Knapp, 1996; Humphrey & Schupp, 2004). This *B. tectorum*-fire cycle concerns ecological and public communities. Breaking the *B. tectorum* - fire cycle through restoration of native communities is pertinent.

Seeding is often used in restoration of these communities, but seeding alone is often not sufficient. If the system is severely disturbed, arbuscular mycorrhizal fungi (AMF) populations may be diminished (Reeves *et al*., 1979; Allen, 1989). AMF are the fungal symbiont of a plant-fungus mutualism termed mycorrhizae (Allen, 1996). This mutualism is common among land plants (Harley & Harley, 1987; Allen, 1996).

In the AMF mutualism, the plant provides carbon to the fungus while the fungus provides soil resources to the plant. The fungus is considered an obligate symbiont requiring carbon from the plant for substantial growth while the plant is considered a facultative symbiont not requiring the fungal symbiont if resource supply is adequate (Gianinazzi-Pearson & Smith 1993; Smith & Read, 1997). The plant-fungus relationship ranges from mutualistic to parasitic. If environmental conditions are favorable for the plant -- i.e. high soil nutrients and moisture -- the AMF may act like a parasite, draining

carbon from the plant while providing little benefit (Bethlenfalvay et al., 1982; Johnson *et al.*, 1997). However, AMF may be necessary for many native perennial species to establish and persist, especially in stressful environments. AMF are beneficial in stressful environment because they can improve the following: nutrient uptake (Chandrashekara *et al.*, 1995; Al-Karaki *et al.*, 1999; Clark & Zeto, 2002), drought tolerance (Allen & Boosalis, 1983; Allen & Allen, 1986; El-Tohamy *et al.*, 1999; Clark & Zeto, 2002; Entry *et al.*, 2002) and disease resistance (Sharma & Johri, 2002) leading to greater plant growth and health.

One of the major benefits of the mycorrhizal symbiosis is enhanced P uptake (Chandrashekara *et al.*, 1995; Mohammad *et al.*, 2004) although they can also increase the uptake of other nutrients such as K, N, Zn, Mg, Cu, and Fe (Al-Karaki *et al.*, 1998; Clark & Zeto, 2002). Phosphorus is a growth-limiting nutrient with low mobility, thus the more absorptive surface area a plant has in the soil, the greater potential P uptake of the plant (Koide, 1993). Mycorrhizae increase P uptake by increasing the absorptive surface area of the root system via an extensive hyphal network (Hetrick, 1991). Mycorrhizal hyphae also explore a greater soil volume and penetrate smaller pores than fine roots and root hairs (Gianinazzi-Pearson & Smith, 1993; Clark & Zeto, 2002). P is transported from the external hyphae or mycelium to internal hyphae and arbuscules in the plant's roots where it is transferred to the host plant (Allen, 1996).

A moderate or high intensity fire can greatly reduce or eliminate AMF propagules near the soil surface (Pattinson *et al.*, 1999), but AMF propagules from nearby unburned areas or from deeper in the soil profile can re-colonize the upper soil layers quickly (Pattinson *et al.*, 1999; Korb *et al.*, 2003). The temporarily reduced or eliminated AMF

population near the soil surface allows non-mycorrhizal and facultative plant symbionts, such as invasive annuals to colonize the area (Reeves *et al.*, 1979; Allen, 1984). Even if AMF propagules are not diminished post-fire, AMF activity may decrease due to a loss of mycorrhizal plants in the system allowing less mycorrhizal dependent species to dominate (O'Dea 2007). Frequent fire can also change AMF species composition (Gibson & Hetrick, 1988) or decrease richness (Eom *et al.*, 1999), which could affect plant species composition due to plant-fungus compatibility (Bever, 1999). The presence of invasive annuals prior to perennial establishment, such as *B. tectorum*, can further alter AMF species composition to favor the invasive(s) and diminish AMF species diversity in native plant roots (Hawkes *et al.*, 2006), possibly shifting the competitive balance in favor of the invasive. Thus, temporary post-fire diminishment of AMF propagules and/or changes in AMF species composition may negatively affect establishment of desirable perennial species and help perpetuate the *B. tectorum* fire cycle.

Despite their potential importance, studies assessing the use of mycorrhizae in *B. tectorum*-invaded areas of the Great Basin are limited. Research is needed that addresses how mycorrhizal inoculum may be used in *B. tectorum* disturbed systems. In particular, it is important to understand how mycorrhizae may affect competition between *B. tectorum* and native grasses. Although not as complete, studies assessing the general response of species to mycorrhizae can provide important complements to competition studies. Several studies have assessed the general response of *B. tectorum* and some Great Basin grasses to mycorrhizae (for example: Allen, 1984, 1988; Trent *et al.*, 1993; Rowe *et al.*, 2007), but literature on some important Great Basin species is lacking. Other mycorrhizal studies have looked at competition between *B. tectorum* and native

grasses (Benjamin & Allen, 1987; Schwab & Loomis 1987; Goodwin, 1992), but these studies are even more limited. It is generally thought that inoculation should favor the more mycorrhizae-dependent species in a system (Allen & Allen, 1990; Hartnett *et al.*, 1993; Hart *et al.*, 2003; Ruotsalainen & Aikio, 2004; Scheublin *et al.*, 2007). However, Schwab & Loomis (1987) found that mycorrhizal benefits shifted from *Pseudoregneria spicatum* to *B. tectorum* as the native outnumbered the invasive. Other studies have found that inoculation favors the less mycorrhizae-dependent species (Marler *et al.*, 1999). The identity of AMF isolates used for inoculation can further influence competitive outcomes (Scheublin *et al.*, 2007).

Since AMF species identities can influence competition, the source of inoculum is important in restoration projects. Either commercial inoculum or local inoculum can be used. The benefit of local inoculum is that the local AMF are more likely adapted to the site, and plant-fungus feedbacks likely have selected beneficial AMF species communities (Lambert *et al.*, 1980; Johnson *et al.*, 1992; Eom *et al.*, 2000). However, if severe disturbances have occurred, the local AMF community may no longer be as beneficial, and the use of commercial inoculum may introduce more beneficial AMF into the system (Powell, 1976, 1977).

In this thesis I will look at the general responses of *B. tectorum*, *P. spicatum* and *Elymus elymoides* to mycorrhizal symbiosis by measuring how the three species' morphology and physiology changes under different P and water availabilities, and intraspecific densities (Chapter 2). The information gathered from the general response study will be used as a baseline to help interpret a competition study in Chapter 3. The competition study will evaluate how the three species respond to local and commercial

inocula under both interspecific and intraspecific competition. I will look at how the species response to each inoculum changes (or does not change) as the identity and number of competitors is altered. In Chapter 4, I will discuss the use of local and commercial inocula in restoration projects, and how ecologists, land managers and the public may evaluate whether they should use mycorrhizae and if mycorrhizae is used, what is the best source for their project(s).

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CHAPTER 2

MYCORRHIZAE AND PLANT PHYSIOLOGY AND MORPHOLOGY

Summary

The introduction of the annual grass *Bromus tectorum* has drastically altered the Great Basin, USA ecosystem. Since the AMF community can be altered by disturbance, the use of inoculum may help improve the AMF composition in these *B. tectorum*-disturbed systems, aiding native perennial establishment and restoration. In this study, two native Great Basin perennial grasses, *Pseudoregneria spicatum* and *Elymus elymoides*, and an exotic invasive annual grass, *B. tectorum*, were examined for their responses to commercial inoculum in a greenhouse pot experiment. Density, phosphorus (P), and water availability were altered to test the effect of different abiotic and biotic stressors on responses to inoculum. Mycorrhizae had subtle effects on growth. Contrary to expectations, *B. tectorum* had the greatest response to mycorrhizae, but the response was often negative, which is not atypical. Mycorrhizal plants of all three species had increased specific root length and reduced leaf area. These unexpected results, along with the lack of a mycorrhizal effect on typical mycorrhizal species response variables such as shoot and root dry mass suggests that soil P was sufficient in both P treatments. An interaction between watering and inoculum treatments may suggest that in this greenhouse system, mycorrhizal plants were using a drought tolerance strategy while non-mycorrhizal plants were using a drought avoidance strategy.

Introduction

The Great Basin has been drastically altered by the invasion of the exotic annual grass *Bromus tectorum* (Stewart & Hull, 1949; Knapp, 1996; Humphrey & Schupp, 2004). Among other traits, *B. tectorum*'s phenology (Rice *et al.,* 1992) and ability to shorten the fire interval and quickly regenerate post-fire, gives it a competitive advantage over native perennials (Wright, 1985; Humphrey & Schupp, 2004).

An important tool for restoring *B. tectorum*-degraded Great Basin ecosystems may be the use of mycorrhizae, a plant-fungus mutualism. Disturbances can greatly alter, the community of arbuscular mycorrhizal fungi (AMF), resulting in declines in abundance (Pattinson *et al.*, 1999), shifts in species composition and diversity (Gibson & Hetrick, 1988; Eom *et al.*, 1999), and/or reductions in the rate of root colonization (de Varennes & Goss, 2007). These changes in the AMF community may have important ramifications for the plant community through plant-fungus feedbacks (Bever, 1999). Although AMF can quickly re-colonize a disturbed site (White *et al.*, 2008), even slight delays in colonization may give invasive species an opportunity to establish and alter the system, including further alterations in the AMF community that favor the invasive (Bever, 1999). In addition, if the invasive is non-mycorrhizal, the AMF population may continue to decline because they lack plant hosts (Allen, 1988). Thus, AMF inoculation of *B. tectorum*-dominated sites may improve the establishment of native perennials.

Mycorrhizae can benefit plant species in a variety of ways. During drought, mycorrhizae can decrease stomatal resistance to water loss and increase drought resistance of plants (Allen & Boosalis, 1983; Allen & Allen, 1986; El-Tohamy *et al.*, 1999; Clark & Zeto, 2002; Entry *et al.*, 2002), by increased water uptake via hyphae

(Ruiz-Lozano & Azcón, 1995; Marulanda *et al.*, 2003), mycorrhizal-mediated improved root conductance (Koide, 1993; Marulanda *et al.*, 2003), or increased root length density (Bryla & Duniway, 1997). Mycorrhizae may also increase water use efficiency (WUE) (Al-Karaki *et al.*, 1998; Ruiz-Lozano *et al.*, 2000; Augé, 2001; Bolandnazar *et al.*, 2007; Querejeta *et al.*, 2007).

Mycorrhizae can also facilitate plant uptake of critical nutrients (Clark & Zeto, 2002), particularly phosphorus (P), and especially in P-depleted soils where plants may have greater dependency on mycorrhizae for growth. Several experiments have shown increased mycorrhizal colonization at decreased soil P levels (Hetrick *et al.*, 1986; Chandrashekara *et al.*, 1995; Al-Karaki & Clark, 1999; El-Tohamy *et al.*, 1999); however, other studies have observed otherwise (Mohammad *et al.*, 2004; Li *et al.*, 2005). To further complicate our understanding, percent colonization does not necessarily correspond with mycorrhizal effectiveness (Ahiabor & Hirata 1994; Smith *et al.*, 2004). That is, mycorrhizae may have a great effect on plant growth and success yet have low root colonization, or vice versa.

Inoculated plants often have higher tissue P concentrations than non-inoculated plants due to the increased P uptake by mycorrhizae (Sharma & Johri, 2002; Singh & Adholeya, 2002; Giri *et al.*, 2005). However, increased P uptake may be offset by increased plant growth resulting in similar P concentrations between inoculated and non-inoculated plants (Al-Karaki *et al.*, 1998; Li *et al.*, 2005). The effect of mycorrhizae on P concentrations can also depend on resource conditions (Al-Karaki *et al.*, 2004).

Mycorrhizal associations can also change the allocation of carbon in the plant, altering root:shoot ratios. However, the effect of mycorrhizae on root: shoot ratios

depends on the plant species and the environment. Mycorrhizal plants may have either increased, decreased, or unchanged root: shoot ratios compared to non-mycorrhizal plants (Allen, 1996; Al-Karaki *et al.*, 1998; Ayres *et al.*, 2006). Because less carbon is needed to maintain mycorrhizal hyphae than to develop extensive root systems, mycorrhizae can increase nutrient uptake by extending the depletion zone of the root system with less carbon cost than needed for roots (Koide, 1993; Allen, 1996). Increased nutrient uptake leads to increased photosynthesis and plant growth (Kwapata & Hall, 1985; Smith & Read, 1997). However, under nutrient rich conditions or during initial growth, the cost of mycorrhizae can be greater than the benefits, reducing plant growth compared to that of non-mycorrhizal plants (Bethlenfalvay *et al.*, 1982; Pandey *et al.*, 2006). Plant growth can also be similar among mycorrhizal and non-mycorrhizal plants depending on resource conditions and colonization levels (Allen & Boosalis, 1983; Kothari *et al.*, 1990).

Mycorrhizae can affect root and shoot morphology of plants as well. Specific root lengths of mycorrhizal-inoculated *Allium porrum* (Berta *et al.*, 1993) and *Gossypium hirsutum* (Price *et al.*, 1989) were reduced compared to controls, presumably due to decreased fine root production (see Kothari *et al.*, 1990). Mycorrhizal effects on root morphologies such as root weights and root lengths may be negligible or reverse directions in more fertile systems (Berta *et al.*, 1993). Greater tillering (Miller *et al.*, 1987; McHugh & Dighton, 2004) and specific leaf area (Snellgrove *et al.*, 1982; Harris *et al.*, 1985; Baas & Kuiper, 1989; Miller *et al.*, 2002) have also been observed in mycorrhizal plants, although the effects of mycorrhizae on shoot structures may depend

on nutrient conditions and carbon demand by the AMF (Kothari *et al.*, 1990; Pandey *et al.*, 2006).

Resource availability and intraspecific density can alter the effect of mycorrhizae on plant morphology and physiology. Mycorrhizae are reported to be less beneficial to plants grown at high densities (Facelli *et al.*, 1999). However, the benefit of mycorrhizae during intraspecific competition can depend on the plant species and P availability (Hartnett *et al.*, 1993; Facelli *et al.*, 1999; Schroeder & Janos, 2004).

If mycorrhizae increase nutrient and water uptake and biomass of mycorrhizal plants, these changes in morphology and physiology may give mycorrhizal plants an advantage over non-mycorrhizal plants. Or as suggested by Allen & Allen (1990) and Hart *et al.* (2003), plants with greater mycorrhizal dependency will gain greater competitive ability relative to less mycorrhizal dependent species.

The present study sought to determine the response of three grasses to mycorrhizal symbiosis: the native perennials *Pseudoregneria spicatum* and *Elymus. elymoides*, and the exotic invasive annual *B. tectorum*. Specifically, I addressed the following 3 questions: (1) What is the effect of mycorrhizae on root: shoot ratios, root dry mass (RDM), shoot dry mass (SDM), specific leaf area (SLA), leaf area (LA), specific root length (SRL), root length, tiller number, total water use efficiency (WUE), shoot WUE, root WUE, water use, and shoot tissue phosphorus (P) concentration and content of the grasses when grown monospecifically at different P levels? (2) Do mycorrhizae reduce water stress of the grasses as measured by carbon isotope discrimination (CID)? (3) Does the effect of mycorrhizae under different phosphorus levels and watering frequencies change with different intraspecific densities?

Materials and Methods

Experimental design

A 3 x 2 x 2 x 2 x 2 factorial pot experiment with four replicates was set up in the U.S. Department of Agriculture Agricultural Research Service Forage and Range Research Laboratory greenhouse using a complete random block design. The five factors were species (*P. spicatum*, *E. elymoides*, or *B. tectorum*), density (6 or 18 plants per pot), inoculum (commercial: AM120 Basin and High Plains Suite or no inoculum), phosphorus (20 or 50 mg P /kg soil), and water (low or high).

Each replicate served as a block to control for potential temperature/humidity gradients in the greenhouse. Each block had a 5 pot x 10 pot arrangement, which minimized edge effects while allowing all pots to fit on greenhouse benches.

Study species

The native grasses *P. spicatum* and *E. elymoides* were selected because of their different life history traits and abilities to compete with *B. tectorum*, and because both are desirable native restoration species. *E. elymoides* is a short-lived, early seral perennial that can compete with *B. tectorum* (Hironaka & Tisdale, 1963; Arredondo *et al.*, 1998; Jones, 1998; Booth *et al.*, 2003; Humphrey & Schupp, 2004). *P. spicatum* is a long-lived, later seral perennial that appears to be less competitive with *B. tectorum* (Aguirre & Johnson, 1991). Thus, these two species represent different successional stages in the Great Basin allowing for a broader study of the effect of mycorrhizae on invasive and native species in the Great Basin. Both the perennials and invasive annual are considered to be facultative mycorrhizal species (Trappe, 1981; Allen, 1988), although *B. tectorum* is considered to be less dependent on mycorrhizae than the perennials (Allen, 1984, 1988)

Low and high water treatments

Pots in the low and high water treatments were watered when the soil water content reached 5-7% or 10-12%, respectively. When watered, all pots were brought back up to field capacity, which was 15% water content. Percent water content was determined by weighing the pots.

Two WUE control pots were added to each replicate. These control pots were filled with soil (equivalent weight to other pots), but did not contain plants. The control pots were used to account for evaporation of water from the soil in water use and WUE calculations.

Pot preparation

Due to the cost and time required to collect soil from a local sagebrush site, 6.6 liter pots (22 cm diameter x 21.5 cm height) were filled with a steam-sterilized 1:1 beach sand and topsoil (sandy loam) mixture and mixed with a cement mixer. Sand and topsoil were purchased from Logan Landscape Products, Logan, UT, USA. The soil had the following chemical properties: pH: 8.18 (saturation paste extract), P: 20 mg/kg soil (sodium bicarbonate method), NO_3: 54.5 mg/kg soil (KCl extraction/ Cd-Reduction method), NH_4: 13.0 mg/kg soil (KCl extraction), and K: 1569 mg/kg soil (sodium bicarbonate method) as determined by the Utah State University Analytical Laboratories.

Each pot was filled with 4.70 kg of soil then 450 mL of inoculum or sterilized terra green (the substrate for the inoculum, 'the control') was layered on top of the soil, and capped with an additional 1.20 kg (~ 4 cm) of soil to help prevent cross contamination. The inoculum was layered rather than mixed throughout the soil in order to ensure root contact with the inoculum and to reduce the amount of inoculum needed.

Commercial inoculum (AM120 Basin and High Plains Suite), donated by Reforestation Technologies International, Salinas, CA, USA, was used. For the phosphorus treatment, half of the pots had 45% superphosphate hand-mixed into the sand:soil to increase the soil P to 50 mg P/kg soil.

P. spicatum (Anatone) and *E. elymoides* seeds were obtained from the U.S. Department of Agriculture Agricultural Research Service Forage and Range Research Laboratory, Logan, UT, USA, and *B. tectorum* seeds were collected from Simpson Springs and Vernon Hills, Tooele County, UT, USA. Seeds were treated with tetramethyl-thiuram disulfide (fungicide) and pre-germinated in germination boxes for 1-2 weeks prior to planting. Seedlings were planted in a regular, circular pattern. For high-density pots, seedlings were planted in two circles, an inner circle of 6 seedlings and an outer circle of 12 seedlings. The low density treatment pots had a circle of 6 seedlings. Pots were watered with a mister for two weeks after which the water treatments began.

Physiological and morphological measurements

To assess whether mycorrhizae mediate water stress of each species, ground tissue samples were analyzed for $^{13}C/^{12}C$ content. All plants from a pot were mixed together in a paper sack and ground using a Cyclotec 1093 sample mill (Tecator, Sweden). The ground sample was re-mixed in a coin envelope and 3 mg were measured out for analysis. Each pot's ground subsample was sent to The Stable Isotope Laboratory at Utah State University, Logan, UT, USA, to analyze the $^{13}C/^{12}C$ content of the leaf tissue. CID was calculated using the discrimination equation in O'Leary (1993).

C_3 plants preferentially take up the lighter carbon isotope, ^{12}C, due to both enzymatic and physical processes. Ribulose bisphosphate carboxylase/oxygenase

(RuBisCO) more readily fixes ^{12}C than ^{13}C. Diffusion gradients also favor the flow of the lighter isotope ^{12}C (O'Leary, 1993). Plants under water stress discriminate less against the heavier isotope and are enriched in ^{13}C. Mycorrhizae may improve leaf water balance and subsequently show greater discrimination against the heavier isotope. In this experiment, water stressed plants were assumed to be the plants in the low water treatment.

The effect(s) of mycorrhizae on P uptake were assessed by measuring P concentration and P content of shoots and comparing P levels between treatments. Ground tissue samples were analyzed by the Soil and Plant Analysis Laboratory at Brigham Young University, Provo, UT, USA, using the nitrate perchlorate method. Inductively coupled plasma (ICP) was used to analyze the extractions.

Additional measured responses to mycorrhizae were % root colonization, SRL, leaf area, SLA, root length, number of tillers, RDM, SDM, root:shoot ratio, water use total WUE, shoot WUE and root WUE. Due to the short time frame of the experiment, plants were harvested and responses measured only at the end of the experiment. All references to water use, root length, leaf area, tiller number, RDM, and SDM means are per plant values.

As a surrogate for harvests, the number of tillers was counted approximately 25 days after the water treatments began (hereafter referred to as mid-point tiller number) and immediately before the shoot harvest at 50 days after the water treatment began (hereafter referred to as final tiller number). A belt-driven leaf area meter was used to measure leaf area. To measure root length, roots were lightly washed, floated in transparent trays containing water, scanned with a flatbed scanner at 300 dpi, and

analyzed using an image analysis program (WinRhizo, Regent Instruments Inc., Quebec City, Canada). To determine dry mass, shoots and roots were oven dried at 60˚ C for 8 days and weighed.

To analyze differences in water use efficiency between treatment combinations, root water use efficiency (root dry mass/water use), shoot water use efficiency (shoot dry mass/water use) and total water use efficiency (root+shoot dry mass/water use) were all calculated.

Mycorrhizal colonization measurements

While harvesting each root mass, a root sample for mycorrhizal quantification weighing 1-2 grams was cut and stored in 50% ethanol. Each sample had four subsamples, two from shallower and two from deeper roots. The dry weight of each sample used for mycorrhizal quantification was estimated and added to the total root weight using each mycorrhizal root sample's fresh weight and the corresponding root mass' fresh weight/dried weight.

Roots for mycorrhizal quantification were stained and cut into ~1 cm pieces using the protocol in Phillips & Hayman (1970). The protocol was optimized for the type of roots being stained and to reduce the use of toxic chemicals. Roots were cleared for 30 minutes and stained for 12 minutes. Lactoglycerol rather than lactophenol was used to store the stained root specimen and in the 0.05% trypan blue staining solution. Hyphal, arbuscular and vesicular colonization was measured using the magnified gridline intersect method and a 400-x magnification lens (Giovannetti & Mosse, 1980; McGonigle et al., 1990).

Statistical analyses

A mixed model 5-way ANOVA was used to determine the effect of each fixed factor combination on each response variable in SAS v 9.1.3 (2003) using the PROC MIXED command. The five fixed explanatory factors were species, density, inoculum, phosphorus, and water, with block as a random factor. The response variables were root:shoot ratio, RDM, SDM, LA, root length, SLA, SRL, mid-point tiller number, final tiller number, CID, shoot tissue P concentrations, shoot tissue P content, water use , shoot WUE, root WUE and total WUE. All analyses of water use, root length, leaf area, tiller number, RDM, and SDM used mean per plant values. Values per plant were calculated as: total pot value/number of surviving plants at harvest.

A 2 x 2 contingency table analysis using the chi-square test in SAS showed that inoculated and non-inoculated pots differed in the presence/absence of mycorrhizae; i.e., that the non-inoculated pots were truly control pots. A 4-way ANOVA using the species, density, phosphorus and watering regime treatments was performed to determine what effects the different treatment combinations had on percent mycorrhizal root colonization of inoculated pots using the PROC MIXED command in SAS v 9.1.3 (2003).

Statistical significance was set at the 0.01 probability level. This decision was based on a desire to use a more severe criterion than the 0.05 probability level for rejecting the null hypothesis due to the very large number of class and response variables used in the study, but without using the excessively conservative sequential Bonferroni method.

The following response variables were transformed as indicated to meet assumptions of normality and homogeneous variance. Percent root colonization, mid-

point tiller number, and LA were square root transformed. Root:shoot ratio, RDM, final tiller number, root WUE, shoot WUE, total WUE, and water use were cube root-transformed. P content, SLA, and SRL were log-transformed. Root length was quarter root-transformed. A MIXED model with reduced heterogeneous variance structure was used for total WUE and shoot WUE to account for unequal variance in species and in both species and density parameters, respectively. Least squared mean comparisons were made for all statistically significant interactions and/or main effects. All least squared means and standard errors were back-transformed for presentation in figures, tables, and the text.

Four data points from the no-inoculum treatment were removed from analysis of all response variables because they had greater than 10% colonization. They were not treated as inoculated because the source of contamination was not known.

Throughout, significant main effects are not discussed when they are part of a significant higher order interaction.

Results

Percent root colonization

The contingency analysis showed that the presence of mycorrhizae in mycorrhizal pots (83/12) was significantly different from non-mycorrhizal pots (15/80) (χ^2=97.445; df = 1; P <0.0001). Fifteen non-inoculated pots contained colonized roots, but 11 of these pots had <10% colonization.

Percent root colonization was significantly affected by species, density, the species x water interaction, the species x density interaction and, the P x density interaction (Table 2.1). The significant species x water interaction shows that *B.*

tectorum had greater root colonization in the high water treatment while root colonization of the perennials was not affected by water treatment (Figure 2.1). In contrast, the significant species x density interaction shows that both *P. spicatum* and *E. elymoides* had significantly greater root colonization in the high density than in the low density treatment, but *B. tectorum* did not respond to density (Figure 2.2). The P x density interaction shows that root colonization was significantly greater when plants were most stressed with the combination of high density and low P, while all other combinations did not differ (Figure 2.3).

Water use, total water use efficiency, shoot water use efficiency, and root water use efficiency

Water use was significantly affected by species, water, density, the species x density interaction, the water x inoculum interaction, and the species x water x P x density interaction (Table 2.2). Although inoculum treatment did not significantly affect water use in either watering treatment, the water x inoculum interaction was significant because in the low water treatment, non-inoculated plants used less water than inoculated plants, where as in the high water treatment non-inoculated plants had greater water use than inoculated plants (Figure 2.4).

Although the species x water x P x density interaction was significant, the most important component of this interaction was the highly significant species x density interaction; water use of *B. tectorum* was significantly greater in the low density than in the high density treatment, while density did not affect water use in the perennials (Figure 2.5). The higher order interaction was created by subtle though almost always insignificant shifts in the effects of density on water use across combinations of species,

P, and water (Figure 2.5); consequently, the importance of the higher order interaction is minimal and it is possibly even spurious.

Total WUE was significantly influenced by species, density, and the species x density interaction (Table 2.2). The significant water x density interaction shows that the perennials had greater total WUE when plant density was low versus high, although this was significant only for *P. spicatum* while *B. tectorum* total WUE did not respond to the density treatment (Figure 2.6).

Shoot WUE was significantly affected by species and density (Table 2.2). Shoot WUE was greater at low density ($3.50e^{-3}$ g/g $\pm$ $3.26e^{-4}$) than at high density ($2.74e^{-3}$ g/g $\pm$ $2.45e^{-4}$). *P. spicatum* ($3.36e^{-3}$ g/g $\pm$ $3.28e^{-4}$) and *E. elymoides* ($3.79e^{-3}$ g/g $\pm$ $3.60e^{-4}$) did not differ but both had significantly greater shoot WUE than did *B. tectorum* ($2.30e^{-3}$ g/g $\pm$ $2.17e^{-4}$).

Root WUE was significantly influenced by species, water, and inoculum (Table 2.2). In contrast to shoot results, *B. tectorum* had significantly greater root WUE ($7.66e^{-4}$ g/g $\pm$ $1.06e^{-4}$) than did the perennials (*P. spicatum*: $5.77e^{-4}$ g/g $\pm$ $8.78e^{-5}$, *E. elymoides*: $4.78e^{-4}$ g/g $\pm$ $7.79e^{-5}$), which did not differ from each other. Root WUE was greater when water was less available (low water: $6.47e^{-4}$ g/g $\pm$ $9.26e^{-5}$, high water: $5.55e^{-4}$ g/g $\pm$ $8.40e^{-5}$) and for non-mycorrhizal plants (non-mycorrhizal plants: $6.49e^{-4}$ g/g $\pm$ $9.30e^{-5}$, mycorrhizal plants: $5.53e^{-4}$ g/g $\pm$ $8.36e^{-5}$).

Root dry mass, specific root length, and root length

RDM was significantly affected by species, density, the species x density interaction and the species x water x P x density interaction (Table 2.2). Although the

species x water x P x density interaction was significant, once again the main component of this 4-way interaction seems to be the highly significant species x density interaction. In fact, this 4-way interaction is very similar to the significant species x water x P x density interaction for water use. *B. tectorum* had a much greater response to the density treatment, with significantly lower RDM at high versus low density, whereas perennial RDM did not generally respond to the density treatment (Figure 2.7). Although there appears to be subtle patterns occurring among the water and P treatments for each species, these patterns are mostly insignificant and do not seem biologically important; further, these results may be spurious.

SRL was significantly affected by species and inoculum (Table 2.2). Similar to RDM, *B. tectorum* had greater SRL than both perennials. *E. elymoides* had greater SRL than *P. spicatum* (Table 2.3). Plants grown with the commercial inoculum had greater SRL than in the no inoculum treatment (Table 2.3).

Per plant RL was significantly influenced by species, density and the species x density interaction (Table 2.2). Similar to the species x density pattern seen within the 4-way interactions for water use and RDM, the significant species x density interaction for RL shows that *B. tectorum* had a much greater response to the density treatment, significantly reducing RL at high versus low density while perennials did not respond to density (Figure 2.8).

Mid-point tiller number, final tiller number, and shoot dry mass

Mid-point tiller number, final tiller number, and SDM were all significantly affected by species, water, density, and the species x density interaction (Table 2.2). Final tiller number was also significantly affected by the species x water interaction

(Table 2.2). Mid-point tiller number was significantly greater in the high water treatment (4.70 tillers/plant ± 0.38) than in the low water treatment (4.27 tillers/plant ± 0.37). *B. tectorum* mid-point tiller number was greater in the low density than in the high density treatment while density did not affect perennial mid-point tiller number which explains the species x density interaction (Figure 2.9).

Final tiller number for the perennials was significantly greater in the high water than in the low water treatment, while water treatments did not differ for *B. tectorum,* which explains the significant species x water interaction (Figure 2.10a). In contrast to mid-point tiller number, final tiller number was greater in low density than in high density treatments for all species, although the reduction in tiller number under high density was much greater for *B. tectorum* than for the perennials, which explains the significant species x density interaction (Figure 2.10b).

SDM was greater in the high water treatment (0.91g ± 0.13) than the low water treatment (0.71g ± 0.10). As with final tiller number, all three species had greater SDM at low density than at high density, but the difference between densities was much greater for *B. tectorum,* yielding the significant species x density interaction (Figure 2.11).

Root:shoot ratios

Root:shoot ratios were significantly affected by species and density (Table 2.4). *B. tectorum* had a significantly greater root:shoot ratio than the perennials, while *P. spicatum* had a significantly greater root:shoot ratio than *E. elymoides* (0.35g/g ± 0.02, 0.17g/g ± 0.01, and 0.14 g/g ± 0.01 respectively). Root:shoot ratios increased with density (low density: 0.17 g/g ± 0.01, high density: 0.24 g/g ± 0.01).

Leaf area and specific leaf area

LA was significantly influenced by species, water, inoculum, density, the water x density interaction, and the species x density interactions (Table 2.4). Non-mycorrhizal plants had greater LA (23.22 $cm^2 \pm 1.62$) than mycorrhizal plants (20.87 $cm^2 \pm 1.53$). As with final tiller number and SDM, all three species had greater LA at low density versus high density, but *B. tectorum* had the greatest response to the density treatment, yielding the significant species x density interaction (Figure 2.12a). In addition, there was a significant interaction between the water and density treatments, with density having a greater effect in the high water than in the low water treatment (Figure 2.12b).

SLA was significantly influenced by species, density, and the species x inoculum interaction (Table 2.4). SLA was greater at low density (177.25 $cm^2/g \pm 12.23$) versus high density (162.46 $cm^2/g \pm 11.23$). *B. tectorum* SLA was significantly greater in the no inoculum treatment than in the commercial inoculum treatment while the perennials did not differ between the inoculum treatments yielding the significant species x inoculum interaction (Figure 2.13).

Shoot tissue phosphorus content, and phosphorus concentration

Shoot tissue P content was significantly affected by species, water, density, and the species x density interaction (Table 2.4). Shoot tissue P content was greater when water was more available (low water: 1.67 mg/plant $\pm$ 0.17, high water: 2.18 mg/plant $\pm$ 0.22). In common with many previous responses, shoot tissue P content was greater at low density for all three species, but *B. tectorum* showed a greater response to the density treatment which reflects its greater biomass response and explains the significant species x density interaction (Figure 2.14).

Shoot tissue P concentration was significantly affected by species, P, density, the species x inoculum interaction and the water x inoculum x P x density interaction (Table 2.4). *B. tectorum* had reduced P concentration in the commercial inoculum treatment relative to the no inoculum treatment while the perennials did not respond to the inoculum treatments, yielding a significant species x inoculum interaction (Figure 2.15). Overall, P concentration was significantly greater in the low density than in the high density treatment, although the effects of density varied subtly in unpredictable ways across combinations of water, inoculum, and P treatments, creating the significant 4-way interaction (Figure 2.16). There is no obvious biologically meaningful interpretation of this higher order interaction.

Carbon isotope discrimination

CID was significantly affected by species, water, inoculum and the water x P x density interaction (Table 2.4). *P. spicatum* (23.60 $\Delta \pm$ 0.08) had greater CID than *E. elymoides* (23.25 $\Delta \pm$ 0.08) and *B. tectorum* (23.18 $\Delta \pm$ 0.08). CID was greater for

mycorrhizal plants (23.26 Δ $\pm$ 0.07) than non-mycorrhizal plants (23.43 Δ $\pm$ 0.07). CID was significantly less for the low water, high P, and low density treatment combination than for the remaining seven treatment combinations which had indistinguishable discrimination values; this explains the significant water x P x density interaction (Figure 2.17).

Discussion

Mycorrhizal effects

P. spicatum had the greatest root colonization, with *E. elymoides* and *B. tectorum* being statistically equivalent. As seen in other studies, percent root colonization does not necessarily correspond with mycorrhizal effect (Ahiabor & Hirata, 1994; Mohammad *et al.*, 2004; Smith *et al.*, 2004; Li *et al.*, 2005); that is, *P. spicatum* did not have a greater response to mycorrhizae as measured by the other response variables in this experiment. Although its response was often negative, *B. tectorum* was the species whose morphology and physiology responded most to mycorrhizae. This is not unexpected because *B. tectorum* is considered a less mycorrhizal dependent species than the perennial grasses. Some studies suggest that *B. tectorum* is non-mycorrhizal when grown only with non-mycorrhizal species, but tends to be mycorrhizal when grown with other mycorrhizal species (Pendleton & Smith, 1983; Reeves *et al.*, 1979). A study conducted by Hawkes *et al.* (2006) found a shift in the belowground fungal community with *B. tectorum* invasion. Compared to non-invaded perennial grass sites, *B. tectorum* invaded sites had a shift in fungal composition from AMF to saprophytic and pathogenic fungi.

Another example of root colonization not being a good predictor of species response to mycorrhizae was the greater root colonization at low P and high density

compared to the other three treatment combinations even though the other response variables did not show a greater effect of mycorrhizae at low P, high density. At least for some species, increased mycorrhizal root colonization at high density may balance out the effect of greater intraspecific competition resulting in no changes in biomass (Schroeder & Janos, 2004). Interestingly soil colonization might be a better predictor of plant responses than the typical root colonization measurements (Augé *et al.*, 2007).

As in other studies (Li *et al.*, 2005), percent root colonization was greater in the lower soil P treatment, but in the present study this occurred only with the additional stress of high density. At high soil P, mycorrhizal colonization in the high density treatment may have been depressed because the mycorrhizae provided little benefit to the plant. In addition, the increased root density in the high density treatment may have facilitated spread of the inoculum (Schroeder & Janos, 2004). Although root colonization of *P. spicatum* and *E. elymoides* did not respond to water level, *B. tectorum* had significantly greater colonization in the high water treatment. Although some previous studies have found greater colonization when water is readily available (Kwapata & Hall, 1985; Al-Karaki *et al.*, 2004), others have also shown that colonization can be reduced when water is readily available (Al-Karaki *et al.*, 1998). The different response of percent root colonization of perennial grasses compared to the invasive grasses demonstrates the different compatibilities between mycorrhizal fungal symbionts and host plants (Al-Karaki *et al.*, 1998).

Mycorrhizae had only subtle, often unexpected, effects on plant growth under the conditions of this greenhouse experiment. Of the 256 effects involving inoculum, only eight were significant. Mycorrhizae affected responses such as P concentration, LA, and

SRL that impact overall growth, but did not affect dry mass or root:shoot ratios. The minimal effect of mycorrhizae on plant physiology and morphology might indicate that the soil P and water availability were sufficient even in the low P and water treatments.

When mycorrhizae affected plant growth, the inoculum effect often interacted with resource availability (phosphorus, water, and density treatments) and/or species identity. The significant 4-way interaction involving water, inoculum, P, and density for P concentration had no evident biological pattern. This was likely a spurious result since the sample size for the 4-way interaction was small and the probability of a type I error very high (Stevens, 1999). Other than this 4-way interaction, neither P nor density interacted with inoculum to affect plant morphology and physiology. P and density did interact to affect percent root colonization. The lack of any interaction between P or density with inoculum contrasts with other studies that have found that mycorrhizae increase competition intensity for certain species and that the effect of mycorrhizae on competition can be altered by phosphorus availability (Hartnett *et al*., 1993; Facelli *et al*., 1999; Schroeder & Janos 2004).

Facelli *et al.* (1999) found that relative competition intensity was significantly greater in mycorrhizal plants. They also found that increasing density had a significantly greater negative effect on mycorrhizal plants than on non-mycorrhizal plants and mycorrhizal benefits were more common at low plant densities. Hartnett *et al.* (1993) found similar results with obligately mycorrhizal *Andropogon gerardii*. In particular, mycorrhizal benefits were greatest at low densities and decreased as density increased, while density had no effect on non-mycorrhizal plants. However, when they added P the intraspecific competition intensity decreased for mycorrhizal plants and increased for

non-mycorrhizal plants. In contrast, they found conflicting results for facultatively mycorrhizal *Elymus canadensis* where neither mycorrhizae nor added P significantly affected intraspecific competition coefficients. Schroeder & Janos (2004) found similar effects of intraspecific competition and P availability on mycorrhizal responses of *Lycopersicon esculentum* and *Zea mays* as Harnett *et al.* (1993) did for *A. gerardii*. However, they also found that greater intraspecific competition significantly alleviated the negative impact of mycorrhizae on *Coriandrum sativum*.

The lack of a mycorrhizal effect on species responses to increased density for *E. canadensis*, *E. elymoides*, *P. spicatum*, and *B. tectorum* and the amelioration of a negative mycorrhizal effect for *C. sativum* may be due to high resource availability. Mycorrhizae are thought to intensify competitive effects because mycorrhizae increase the plant's accessibility to nutrients such as P. As plants become denser, mycorrhizal plants have increased overlap of P depletion zones compared to non-mycorrhizal plants. However, if nutrients such as P are not limiting, mycorrhizae may not significantly increase overlap of nutrient depletion zones, even at high density, resulting in no mycorrhizal effect on intraspecific competition, or vice versa (see Facelli *et al.*, 1999; Schroeder & Janos, 2004). Further if water is not limiting, mycorrhizae may not impact intraspecific competition. The failure of P addition and the low water treatment to affect plant biomass indicates that P and water were not limiting.

The significant species x inoculum interactions showed that *B. tectorum* in some cases responded differently to mycorrhizae than the perennials i.e. P concentration and SLA. Also, the influences of water and density on percent root colonization were different for the invasive compared to the natives. *B. tectorum* P concentration and SLA

responded differently to mycorrhizae than they did for the perennials. For the invasive, both were reduced in the presence of mycorrhizae while the perennials did not respond to mycorrhizae presence. Other studies have also shown a negative response by *B. tectorum* to mycorrhizae (Schwab & Loomis, 1987; Allen, 1988). *B. tectorum* is an annual that often colonizes disturbed areas that are low in inoculum, so the perennial grasses are expected to have a greater dependency on mycorrhizae (Allen, 1984, 1988).

The neutral effect of mycorrhizae on perennial SLA and negative effect on *B. tectorum* may indicate that soil P and water levels were too elevated for plants to benefit from inoculation. Other studies have found no mycorrhizal effect on SLA when soil P is high (Kothari *et al.*, 1990), whereas when P is deficient, mycorrhizal plants tend to have greater SLA (Snellgrove *et al.*, 1982; Harris *et al.*, 1985). Another indicator of high resource availability was reduced leaf area in inoculated plants.

Since inoculation did not affect SDM and P content of *B. tectorum*, the reduced P concentration of inoculated *B. tectorum* cannot be attributed to the dilution effect (Jarrell & Beverly, 1981). It is possible that commercial inoculum inhibits P uptake in *B. tectorum*. Bethlenfalvay *et al.* (1982) found that control soybean plant shoots had greater percent P than mycorrhizal plants. They ascribe this to competition for P between the AMF and the host's roots. However, they supported this explanation with lower shoot:root ratios of mycorrhizal plants, which was not true in the present study.

The P content data shows that *B. tectorum's* P uptake was greater than that of the perennials. Thus, *B. tectorum* was depleting the soil P in its root zone at a greater rate than were the perennials via its significantly greater root length and SRL (Ayres *et al.*, 2006). The high density of *B. tectorum* roots and its higher P uptake may have caused

mycorrhizae to act as a competitor for P (see Crush, 1973). If *B. tectorum* roots were dense enough, the mycorrhizae would be sequestering P from the same area as *B. tectorum*'s fine roots. Furthermore, if P and water were not limiting, greater access by mycorrhizae to P would have been unnecessary (Koide, 1993). This may have resulted in reduced shoot P concentration for mycorrhizal *B. tectorum* plants. The negative affect of mycorrhizal fungi on *B. tectorum* P concentration is evidence that the AMF were acting more like a parasite than a mutualist when associating with the invasive.

However, the P content data do not support the explanation that mycorrhizae were competing for P, because mycorrhizal *B. tectorum* did not have lower P content than non-mycorrhizal *B. tectorum*. The lack of a mycorrhizal effect on P content was likely interconnected with the lack of a mycorrhizal effect on RDM and SDM. According to Koide (1993), "All else being equal, plants with high rates of growth have greater nutrient demands than those with lower rates." Thus for a given species, plants of similar dry masses, grown under comparable environmental conditions should have similar nutrient uptake.

Inoculation reduced root WUE. Mycorrhizal plants may be less efficient at turning water into root biomass because the carbon initially allocated to roots was going towards mycorrhizal hyphae (Gianinazzi-Pearson & Smith, 1993; Wright *et al.*, 1998; Miller *et al.*, 2002) or lost through root respiration (Koide, 1993). In contrast, inoculation did not affect total WUE or shoot WUE. Inoculation could have increased photosynthesis and stomatal conductance to the same degree resulting in similar total WUE and shoot WUE (Querejeta *et al.*, 2003, 2007).

Inoculated plants had increased SRL compared to non-inoculated plants. If resource conditions are beneficial for mycorrhizal associations, inoculation should decrease fine root production (Kothari *et al.*, 1990). Since mycorrhizal hyphae are essentially functioning as fine roots, but with greater absorptive surface area and accessibility to soil resources (Allen, 1996). However, mycorrhizae can have varying effects on different species in different environments (Berta *et al.*, 1993). The greater production of fine roots in mycorrhizal plants again may indicate that soil P and/or water availability was sufficiently high.

The water use results showed that water uptake by inoculated plants depended on water conditions. Compared to non-inoculated plants, inoculated plants had greater water use in the low water treatment, but less water use in the high water treatment. These results may indicate that mycorrhizae were increasing drought resistance of plants, allowing them to maintain stomatal conductance to water vapor and photosynthesis, where as non-mycorrhizal plants were avoiding drought by closing stomata (Davies *et al.*, 1992; Augé, 2001; Augé *et al.*, 2007). That is, non-mycorrhizal plants decreased water use when water was less available (closing their stomata) whereas mycorrhizal plants maintained a similar level of water use when water stressed compared to when water was more available (maintained stomatal aperture). The fact that mycorrhizal plants maintained stomatal aperture is supported by the CID data where mycorrhizal plants had greater CID than non-mycorrhizal plants. The mechanism for maintaining water use by water stressed mycorrhizal plants could be due to (1) the greater absorptive surface area and access of the mycorrhizal hyphae to water (2) greater root-soil contact and thus better root conductivity of mycorrhizal plants in dry soil and/or (3) greater water availability of

colonized soil due to greater soil aggregation compared to soils lacking mycorrhizae (Davies *et al.*, 1992; Auge, 2001).

The importance of mycorrhizae for nutrient uptake may be intensified when water availability is low. Nutrients are less accessible when soil water content is low, and mycorrhizae may facilitate access to them by reducing diffusion distances. Mycorrhizae may also increase water uptake during times of water stress. Thus, when water and nutrients are more available, the benefits of mycorrhizae may be negligible, but the association may be maintained due to its advantage during times of resource stress (Koide, 1993; Allen, 1996). For perennials, there was a non-significant tendency for greater root colonization in the low water treatment, and root colonization was significantly greater in the high density and low P treatment combinations, possibly indicating a greater reliance on mycorrhizae for water and nutrient uptake under stress (Marulanda *et al.*, 2003). However, nutrient and water stress was not great enough to cause drastic changes in plant morphology and physiology.

Although the effect of the watering treatments on CID indicates that water stress occurred in the low water treatment, this treatment may not have been severe enough to cause significant changes in stomatal conductance and/or water use by mycorrhizal plants. The effect of mycorrhizal hyphae on soil water potential for stomatal closure can depend on water stress severity (Augé *et al.*, 2003, 2007).

The overall negative or neutral response of each species may indicate that mycorrhizae were functioning as an intermediate between mutualists and parasites. The only response variables indicative of a positive effect by mycorrhizae were CID and possibly water use indicating that both P treatments had sufficient P and severe water

stress did not occur. Soil P and water levels were high enough to make "the mycorrhizae superfluous, but not so high as to inhibit infection" (Bethlenfalvay *et al.*, 1982). The rational that soil P was too high for positive mycorrhizal effects is further supported by a subsequent greenhouse experiment where soil P was lower and mycorrhizal effects were more consistently beneficial and significant (Chapter 3).

Further intensifying the high soil P and water effects may be the lack of commercial inoculum's adaptability to greenhouse/species conditions. Although not traditionally thought to have host specificity, plant responses to different AMF can vary greatly depending on the time of year, plant developmental stage and the environmental conditions (Ferrol *et al.*, 2004; Querejeta *et al.*, 2007). By using an AMF mixture for inoculation -- the AM120 Basin and High Plains Suite -- there should have been a better chance of having a good fungal-plant pairing for the given environment.

Non-mycorrhizal effects

Both RDM and water use had significant species x water x P x density interactions. The 4-way interactions for the two responses were complementary in that when water use was greater, RDM was greater. These 4-way interactions clearly show that *B. tectorum* had greater water use, RDM and plasticity (greater response to the density treatment) than did the perennials. Even though these 4-way interactions were statistically significant, they do not appear to be biological significant; significance was likely due to the small sample sizes for the 4-way interactions which greatly increases the probability of a type I error (Stevens, 1999). For both RDM and water use, the species x density interaction was the only significant lower order interaction that was part of the 4-way interaction. *B. tectorum* responded to the density treatment by decreasing water use

and RDM when intraspecific competition was greater while the perennials did not respond to density. This is further evidence that root growth and water use of this annual is more plastic in response to resources than the perennials.

Total WUE and shoot WUE were greater for the perennials than for *B. tectorum*. All three species had greater shoot WUE at low density versus high density, but only the perennials had greater total WUE at low density. The greater total WUE of the perennials and *B. tectorum's* lack of response to the density treatment may indicate that *B. tectorum* had neared the growth carrying capacity within the pots; that is, it was utilizing water but was accumulating biomass because other soil resources were low and limiting growth. When WUE measurements (total, shoot, and root) were taken, *B. tectorum's* growth likely had already plateaued. If WUE measurements were taken when *B. tectorum* was more actively growing, WUE may have been higher. The greater leaf area of *B. tectorum* would also have contributed to less WUE, especially if *B. tectorum* had approached carrying capacity.

Root WUE of *B. tectorum* was greater than that of the perennials, possibly indicating that *B. tectorum* was more efficient at root growth than the perennials; that is, *B. tectorum* used less water per gram of root produced. Root:shoot ratios show that compared to the perennials, *B. tectorum* was allocating more carbon into RDM relative to SDM. The species' root:shoot ratios and root lengths are evidence of greater intraspecific competition for soil resources for *B. tectorum* than for the perennials (Miller *et al.*, 2002).

B. tectorum's significantly greater water use than that of the perennials compensated for its lower total WUE and shoot WUE resulting in *B. tectorum* having greater dry mass than the perennials. In addition, mid-point and final tiller numbers, leaf

area, root length, shoot tissue P content, water use, RDM, and SDM further show *B. tectorum's* greater growth plasticity in response to resource variability compared to the perennials. All eight response variables increased dramatically when intraspecific competition was lower for *B. tectorum*, but for the perennials the responses to density were comparatively minimal or nonexistent. *B. tectorum*'s growth plasticity is well-documented (e.g. Hulbert, 1955; Rice *et al.*, 1992).

Half-way through the experiment, *B. tectorum* had a greater tiller number in the high water treatment compared to the low water treatment, but this difference had disappeared by the end of the experiment, further indicating that *B. tectorum* growth had reached a carrying capacity within the pots. *B. tectorum* is known to have a greater relative growth rate than select perennial grasses (Arredondo *et al.*, 1998), so it is not surprising that this annual would have reached a carrying capacity within pots before the perennial grasses.

B. tectorum also had greater SRL, SDM, RDM, P content, LA, SLA, tiller production, and root length than did the perennials. Due to the short time frame of the experiment and the faster growth rate of *B. tectorum*, it is not surprising that the annual, had greater dry mass and tiller production than the perennials. The differences in RDM, SDM, SRL, root length, SLA, and LA between the invasive and the perennials are typical (Arrendondo *et al.*, 1998; Arrendondo & Johnson, 1999). The greater P content of *B. tectorum* relative to the perennials is consistent with the idea that plants with higher growth rates tend to have greater P uptake (Koide, 1993).

P. spicatum had significantly greater CID than *E. elymoides* and *B. tectorum.* Thus, at least in this greenhouse setting, a species with greater CID does not necessarily

have lower WUE or greater water use, dry mass, or tiller number than do other species with lower CID.

Since water stress should be greatest at high density and low water, stomatal aperture should be reduced under these conditions, resulting in reduced CID (O'Leary, 1993). Looking at the significant water x P x density interaction in this experiment, reduced CID (reduced stomatal aperture) only occurred for the low water, high P, low density treatment combination. In the high soil P treatment, shoot P concentration was greater than in the low soil P treatment. In the low density treatment, shoot P concentration was greater than in the high density treatment. Radin (1984) found that plants with high leaf P concentrations had less sensitivity to abscisic acid (ABA) induced stomatal closing during water stress. Thus, since CID tends to be greater with greater stomatal aperture, high leaf P concentrations should increase CID during water stress. Based on Radin's findings, in my greenhouse experiment, I would expect that if there was a differential response to P and density in the low water treatment (water stress) there would be a reduction in CID in the low P treatment and high density treatment. However, in this study the reverse occurred, CID was reduced in the high P and low density treatment in the low water treatment.

Another possibility is that when drought stressed (low water treatment) the plants in the high P and low density treatment produced drought conditions for themselves more often than the plants in the other P x density treatment combinations by having greater leaf area. That is, since the pots were watered when they reached a target soil water content (5-7% for the low water treatment) the plants grown with higher P availability and less intraspecific competition reached the threshold for watering more often than the

other plants. Plants with larger leaf areas are more negatively affected by low soil water and have reduced carbon isotope discrimination. In this experiment, plants in the low density treatment had greater leaf area than those in the high density treatment, but leaf area was not significantly affected by P level. I do not have a scientific explanation for the significant water x P x density interaction for CID.

When water availability was greater -- high water treatment or low density treatment -- tiller production, shoot WUE, root WUE, CID, P concentration, SLA, SDM and P content were greater and root:shoot ratios were lower compared to when water was less available. When P availability was greater -- high P treatment -- P concentration was greater. Greater water availability would also have increased plant access to P by diffusion (Koide, 1993) which may have led to greater tiller production, P concentration and content and SDM in the high water and low density treatments through improved nutrient status. Thus when resource availability was greater, plants had greater growth, better nutrient status and better leaf-water relations.

Leaf area and SDM were greater when intraspecific competition was lower and thus water more available. In the low density treatment, leaf area increased when more water was available, while in the high density treatment leaf area was similar between water treatments. This result could indicate that self shading as well as soil resource competition was limiting leaf area.

In conclusioni, for experiment 1 the soil nutrient and water levels were sufficient rendering the mycorrhizae superfluous. *B. tectorum* had the greatest response to mycorrhizae, but its response was often negative. Mycorrhizal and non-mycorrhizal plants demonstrated different drought resistance strategies. Mycorrhizal plants

demonstrated drought tolerance while non-mycorrhizal plants demonstrated drought avoidance.

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Table 2.1 P-values for fixed effects of percent root colonization, a measure of the percent of the root system colonized by mycorrhizae. Significant p-values at the 0.01 level are indicated in bold.

Fixed Effect	P-value
Species	**0.0013**
Water	0.8494
P	0.0318
Density	**<0.0001**
Species x Water	**0.0016**
Species x P	0.4716
Species x Density	**0.0059**
Water x P	0.5048
P x Density	**0.0008**
Water x Density	0.3027
Species x Water x P	0.2670
Species x Water x Density	0.9053
Species x P x Density	0.9812
Water x P x Density	0.6279
Species x Water x P x Density	0.0555

Table 2.2 P-values for fixed effects (Species, Sp; Water, W; Inoculum, I; Phosphorus, P; and Density, D) of water use per plant, total water use efficiency (WUE), shoot WUE, root WUE, root dry mass (RDM) per plant, specific root length (SRL), root length (RL) per plant, shoot dry mass (SDM) per plant, mid-point tiller #, and final tiller #. Significant p-values at the 0.01 level are indicated in bold.

Fixed Effect	Water Use Per Plant	Total WUE	Shoot WUE	Root WUE	RDM per plant	SRL	RL per plant	SDM per plant	Mid-point Tiller #	Final Tiller #
Sp	**<0.0001**	**<0.001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
W	**0.0002**	0.6668	0.8918	**0.0082**	0.0174	0.7038	0.1096	**<0.0001**	**0.0028**	**<0.0001**
I	0.8804	0.1525	0.3591	**0.0059**	0.0272	**0.0002**	0.8812	0.5033	0.1705	0.0442
P	0.5363	0.8864	0.8575	0.9123	0.5311	0.8902	0.6123	0.4598	0.4218	0.4071
D	**<0.0001**	**0.0004**	**<0.0001**	0.1191	**<0.0001**	0.3529	**<0.0001**	**<0.0001**	**<0.0001**	**<0.001**
Sp x W	0.8582	0.3635	0.3968	0.3136	0.5315	0.9818	0.6539	0.0779	0.6887	**0.0020**
Sp x I	0.7050	0.2366	0.1395	0.3186	0.2392	0.2988	0.0896	0.1818	0.7500	0.3146
Sp x P	0.7892	0.4204	0.3625	0.8217	0.0678	0.5969	0.1482	0.8786	0.5177	0.8661
Sp x D	**<0.0001**	**0.0049**	0.0106	0.5182	**<0.0001**	0.1211	**<0.0001**	**<0.0001**	**<0.0001**	**<0.001**
W x I	**0.0015**	0.0349	0.0482	0.0479	0.5007	0.2716	0.3859	0.2567	0.3443	0.9709
W x P	0.7227	0.3531	0.5441	0.0423	0.8338	0.0454	0.0960	0.7047	0.9288	0.6052
W x D	0.6482	0.6749	0.8375	0.1865	0.5587	0.7412	0.8677	0.5247	0.0798	0.2232
I x P	0.7771	0.8453	0.9226	0.6664	0.7865	0.5102	0.3634	0.7945	0.9126	0.7066
I x D	0.6393	0.8934	0.6676	0.0936	0.6822	0.0215	0.4665	0.2901	0.5033	0.2998
P x D	0.9076	0.9302	0.6703	0.1270	0.1465	0.8976	0.2046	0.5706	0.2242	0.2965
Sp x W x I	0.6537	0.1460	0.2170	0.1558	0.8642	0.2329	0.6157	0.7041	0.5937	0.8694
Sp x W x P	0.3617	0.2219	0.2373	0.6007	0.2971	0.0776	0.8508	0.6985	0.9157	0.5022
Sp x W x D	0.3027	0.4053	0.5121	0.3046	0.0446	0.7309	0.1427	0.1141	0.0368	0.1560
Sp x I x P	0.5392	0.6487	0.7858	0.1581	0.7043	0.7136	0.9049	0.3857	0.4032	0.4925
Sp x I x D	0.9235	0.7308	0.7737	0.6732	0.3951	0.1438	0.6224	0.7547	0.4201	0.2951
Sp x P x D	0.2652	0.2285	0.3239	0.0695	0.9872	0.3505	0.5616	0.1882	0.8934	0.8371
W x I x P	0.2312	0.5576	0.4351	0.8278	0.7469	0.6048	0.8464	0.6658	0.9325	0.7292
W x I x D	0.2258	0.0338	0.0328	0.4933	0.6630	0.9901	0.5580	0.4875	0.8046	0.3864
W x P x D	0.2505	0.6547	0.3314	0.0698	0.6363	0.4225	0.7714	0.0311	0.5218	0.5829
I x P x D	0.2438	0.1526	0.2556	0.0333	0.4626	0.6754	0.1203	0.0610	0.4693	0.0109
Sp x W x I x P	0.5057	0.2543	0.2652	0.5473	0.7996	0.5530	0.9627	0.9711	0.4307	0.5526
Sp x W x I x D	0.5002	0.0847	0.1155	0.5111	0.8401	0.7185	0.3261	0.7208	0.7834	0.1445
Sp x W x P x D	**0.0004**	0.0289	0.0164	0.8137	**0.0020**	0.1147	0.0960	0.4049	0.1041	0.0289
Sp x I x P x D	0.4656	0.2359	0.2992	0.5297	0.4451	0.2384	0.6312	0.1826	0.4841	0.4114
W x I x P x D	0.8104	0.8448	0.8297	0.9252	0.1721	0.0406	0.5270	0.2234	0.1188	0.3099
Sp x W x I x P x D	0.8400	0.6157	0.7221	0.2520	0.6792	0.0317	0.8448	0.4486	0.4738	0.0921

Table 2.3 Specific root lengths (cm^2/g) for species and inoculum treatments. Significant differences at the 0.01 significance level within a given treatment are indicated by different letters.

Fixed Effects	LSMean	Standard Error
Species		
B. tectorum	15632.80[a]	1470.00
P. spicatum	9156.27[c]	860.32
E. elymoides	12214.60[b]	1147.47
Inoculum		
No Inoculum	11510.70[b]	1072.94
Commercial Inoculum	12608.20[a]	1173.35

Table 2.4 P-values for fixed effects (Species, Sp; Water, W; Inoculum, I; Phosphorus, P; and Density, D) of root:shoot ratios, leaf area (LA), specific leaf area (SLA), P content per plant, P concentration, and carbon isotope discrimination (CID). Significant p-values at the 0.01 level are indicated in bold.

Fixed Effect	Root: shoot ratio	LA	SLA	P content per plant	P concentration	CID
Sp	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
W	0.1057	**<0.0001**	0.6054	**<0.0001**	0.5781	**0.0017**
I	0.0183	**0.0063**	0.2470	0.0850	0.0280	**0.0015**
P	0.9174	0.2196	0.1676	0.0242	**0.0023**	0.1178
D	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.0388
Sp x W	0.3585	0.6982	0.2177	0.0428	0.9463	0.0385
Sp x I	0.0722	0.1598	**<0.0001**	0.7181	**0.0006**	0.0559
Sp x P	0.0262	0.9972	0.7120	0.7012	0.1485	0.5410
Sp x D	0.7352	**<0.0001**	0.0255	**<0.0001**	0.0130	0.6623
W x I	0.7345	0.9588	0.8965	0.6005	0.4106	0.4106
W x P	0.2577	0.5310	0.1999	0.8585	0.3495	0.2637
W x D	0.0614	**0.0051**	0.2470	0.6975	0.3392	0.4511
I x P	0.6074	0.4728	0.4324	0.6358	0.6722	0.0813
I x D	0.3203	0.6382	0.1035	0.6049	0.2072	0.6580
P x D	0.3975	0.3448	0.2691	0.4775	0.9484	0.4965
Sp x W x I	0.6726	0.2655	0.0146	0.9702	0.5695	0.2251
Sp x W x P	0.9181	0.7583	0.7293	0.9396	0.1094	0.7051
Sp x W x D	0.9489	0.0944	0.3748	0.4185	0.0761	0.6060
Sp x I x P	0.8161	0.5413	0.9046	0.2969	0.5009	0.3775
Sp x I x D	0.8983	0.3770	0.8455	0.8885	0.2621	0.8543
Sp x P x D	0.6856	0.1161	0.9240	0.3038	0.4433	0.3927
W x I x P	0.1386	0.8729	0.5296	0.5593	0.4355	0.6474
W x I x D	0.6967	0.8954	0.8444	0.3349	0.7538	0.1905
W x P x D	0.0301	0.4311	0.6928	0.0950	0.0480	**0.0003**
I x P x D	0.9595	0.2982	0.2494	0.0722	0.9444	0.0297
Sp x W x I x P	0.8763	0.8548	0.4595	0.8351	0.4300	0.4968
Sp x W x I x D	0.8691	0.6422	0.9044	0.8371	0.3672	0.1899
Sp x W x P x D	0.0139	0.2742	0.0961	0.3293	0.6319	0.1691
Sp x I x P x D	0.9477	0.7810	0.6749	0.0853	0.1686	0.0486
W x I x P x D	0.4255	0.9670	0.1211	0.9214	**0.0008**	0.6366
S x W x I x P x D	0.5917	0.2679	0.3004	0.4801	0.5781	0.3150

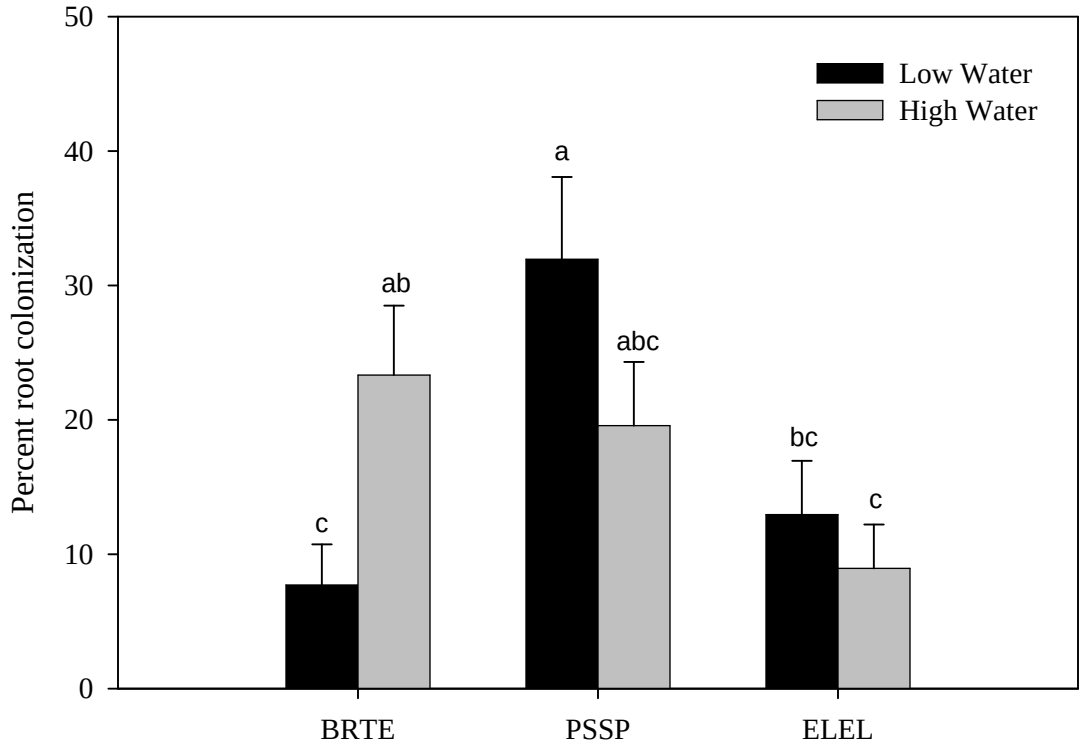

Figure 2.1 The effect of water on percent root colonization across, a measure of the percent of the root system colonized by mycorrhizae, of *B. tectorum* (BRTE), *P. spicatum* (PSSP), and *E. elymoides* (ELEL). Bars represent means of four replicates with error bars representing + 1 standard error. Significant differences among treatments are indicated by different letters ($P < 0.01$).

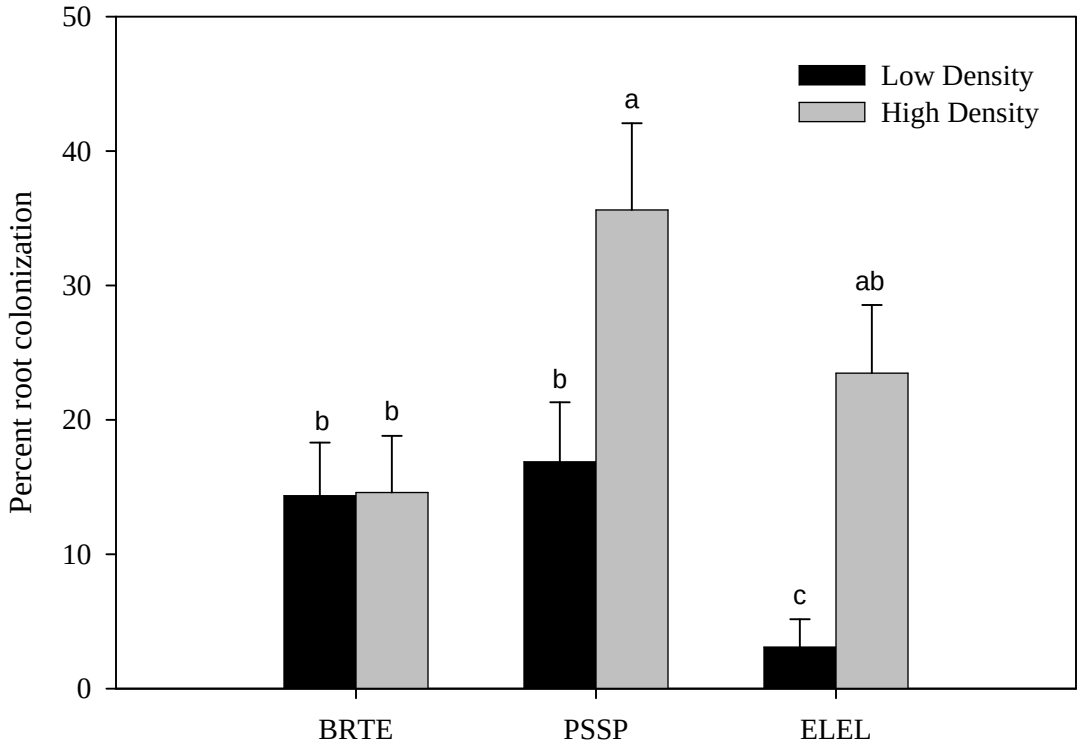

Figure 2.2 The effect of density on percent root length colonization, a measure of the percent of the root system colonized by mycorrhizae, of *B. tectorum* (BRTE), *P. spicatum* (PSSP), and *E. elymoides* (ELEL). Bars represent means of four replicates with error bars representing + 1 standard error. Significant differences among treatments are indicated by different letters ($P < 0.01$).

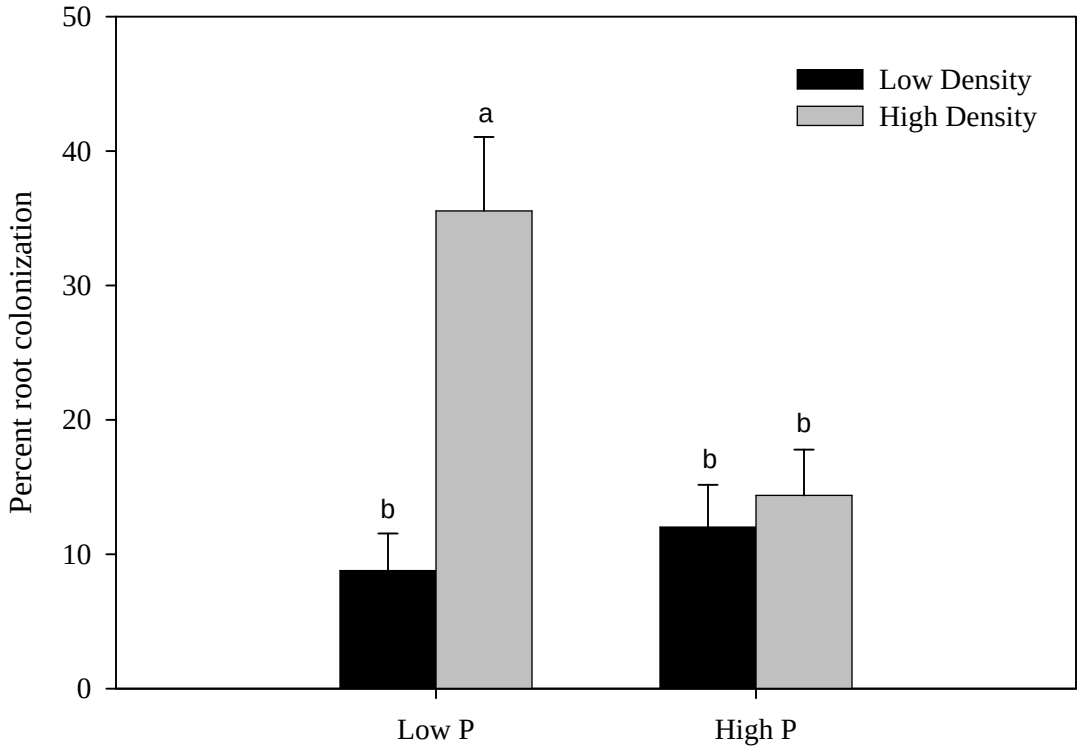

Figure 2.3 The effect of phosphorus, and density on percent root colonization across all three grass species. Percent root colonization is a measure of the percent of the root system colonized by mycorrhizae. Bars represent means for four replicates with error bars representing + 1 standard error. Percent root colonization is a measure of the percent of the root system colonized by mycorrhizae. Significant differences among treatments are indicated by different letters ($P < 0.01$).

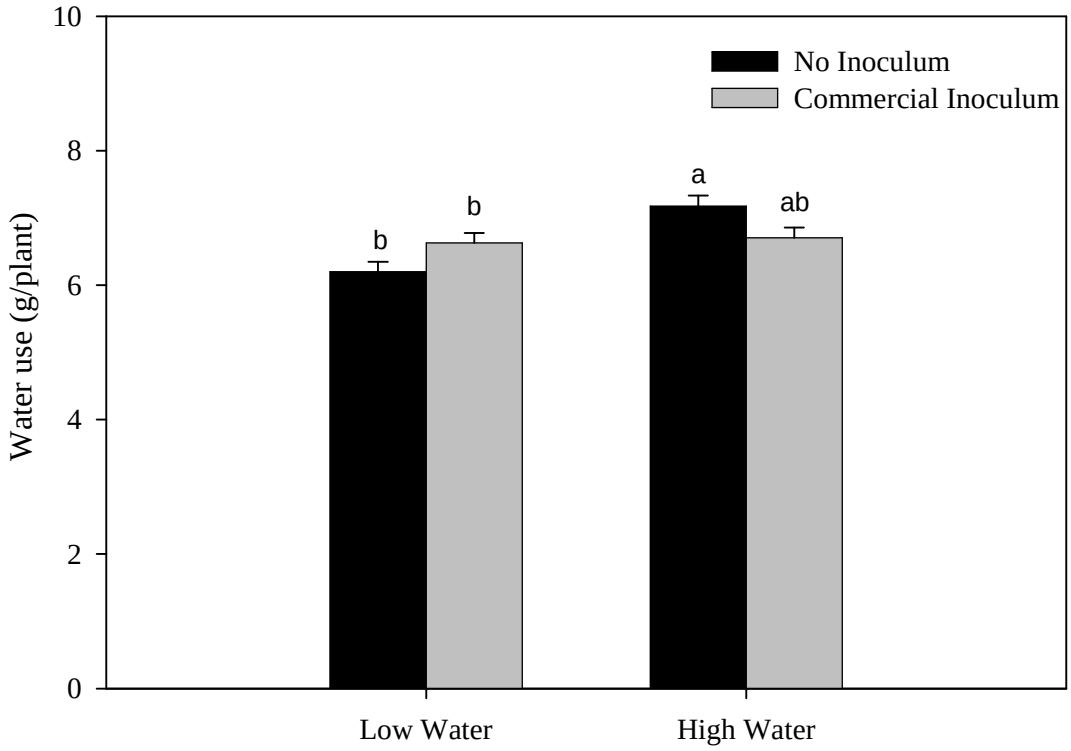

Figure 2.4 The effect of water, and inoculum on water use across all three grass species. Bars represent means of four replicates with error bars representing + 1 standard error. Water use was calculated as: (total grams of water applied to pots − evaporative loss)/number of surviving plants at harvest. Significant differences among treatments are indicated by different letters ($P < 0.01$).

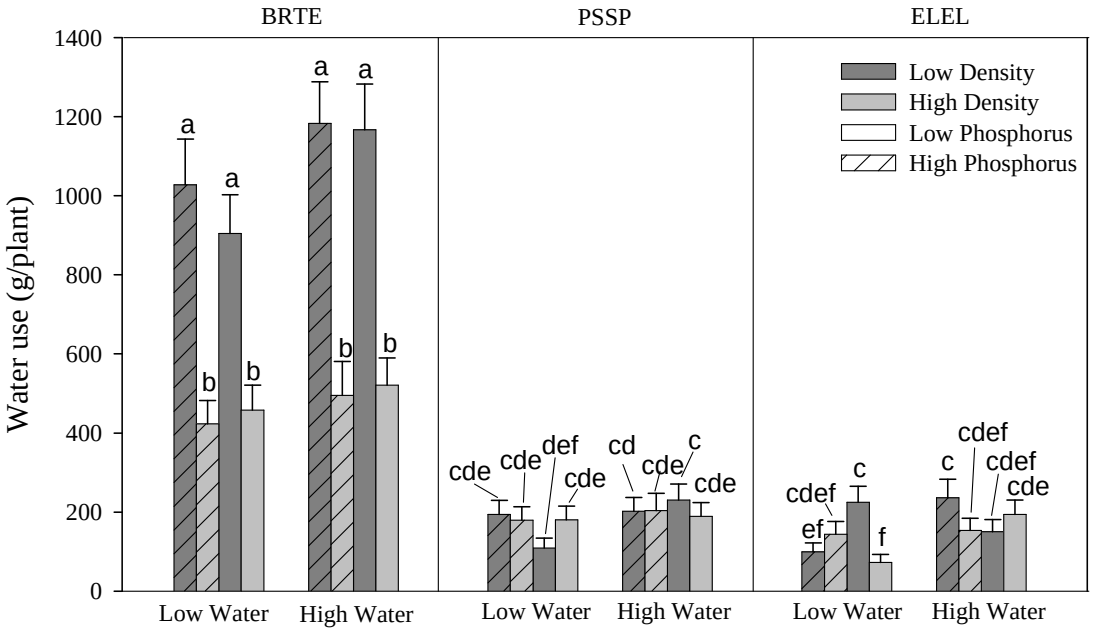

Figure 2.5 The effect of water, P, and density on water use of *B. tectorum* (BRTE), *P. spicatum* (PSSP), and *E. elymoides* (ELEL). Bars represent means of four replicates with error bars representing + 1 standard error. Water use was calculated as: (total grams of water applied to pots − evaporative loss)/# of surviving plants at harvest. Significant differences among treatments are indicated by different letters ($P < 0.01$).

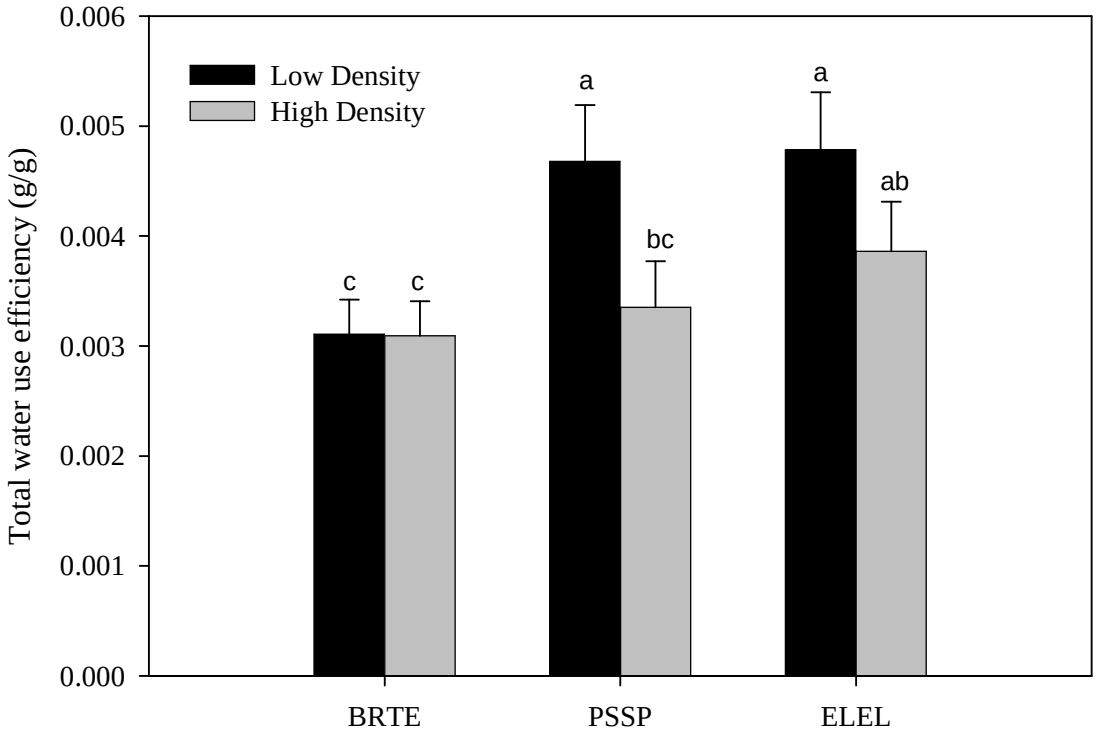

Figure 2.6 The effect of density on total water use efficiency of *B. tectorum* (BRTE), *P. spicatum* (PSSP), and *E. elymoides* (ELEL). Bars represent means of four replicates with error bars representing + 1 standard error. Total water use efficiency was calculated as: total dry mass of plant/grams of water use. Significant differences among treatments are indicated by different letters ($P < 0.01$).

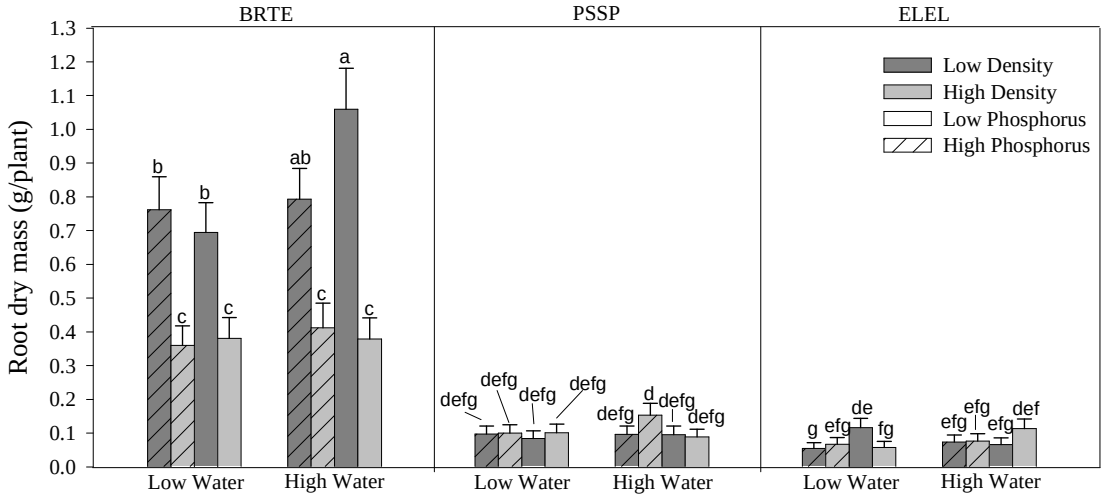

Figure 2.7 The effect of water, P, and density on root dry mass of *B. tectorum* (BRTE), *P. spicatum* (PSSP), and *E. elymoides* (ELEL). Bars represent means of four replicates with error bars representing + 1 standard error. Significant differences among treatments are indicated by different letters ($P < 0.01$).

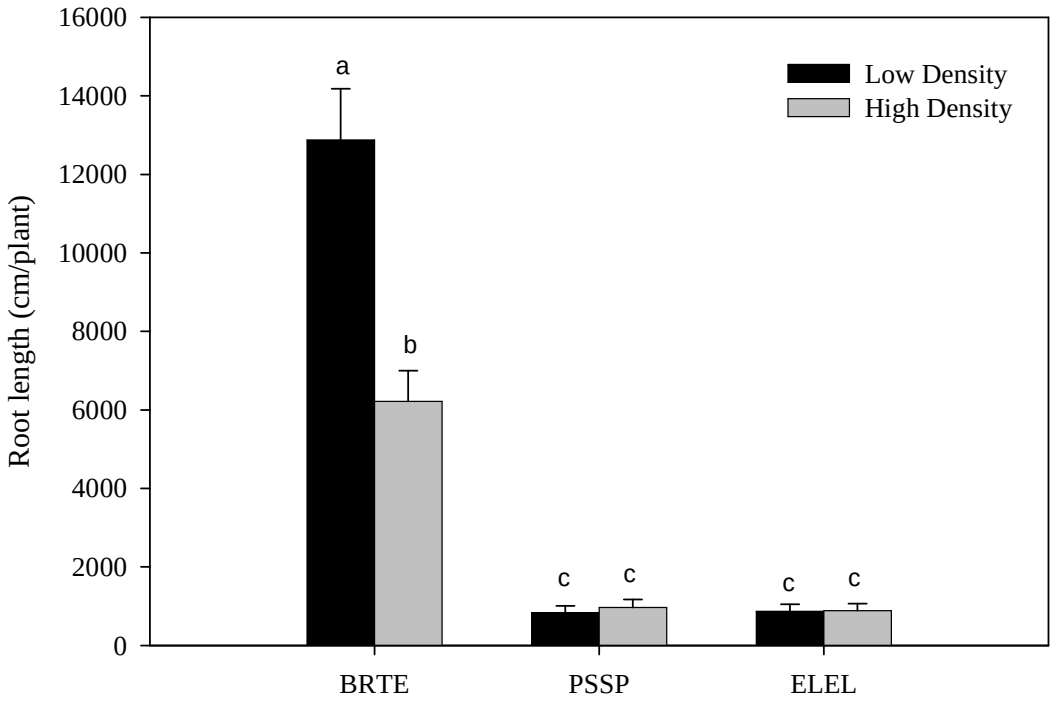

Figure 2.8 The effect of density on root length of *B. tectorum* (BRTE), *P. spicatum* (PSSP), and *E. elymoides* (ELEL). Bars represent means of four replicates with error bars representing + 1 standard error. Significant differences among treatments are indicated by different letters ($P < 0.01$).

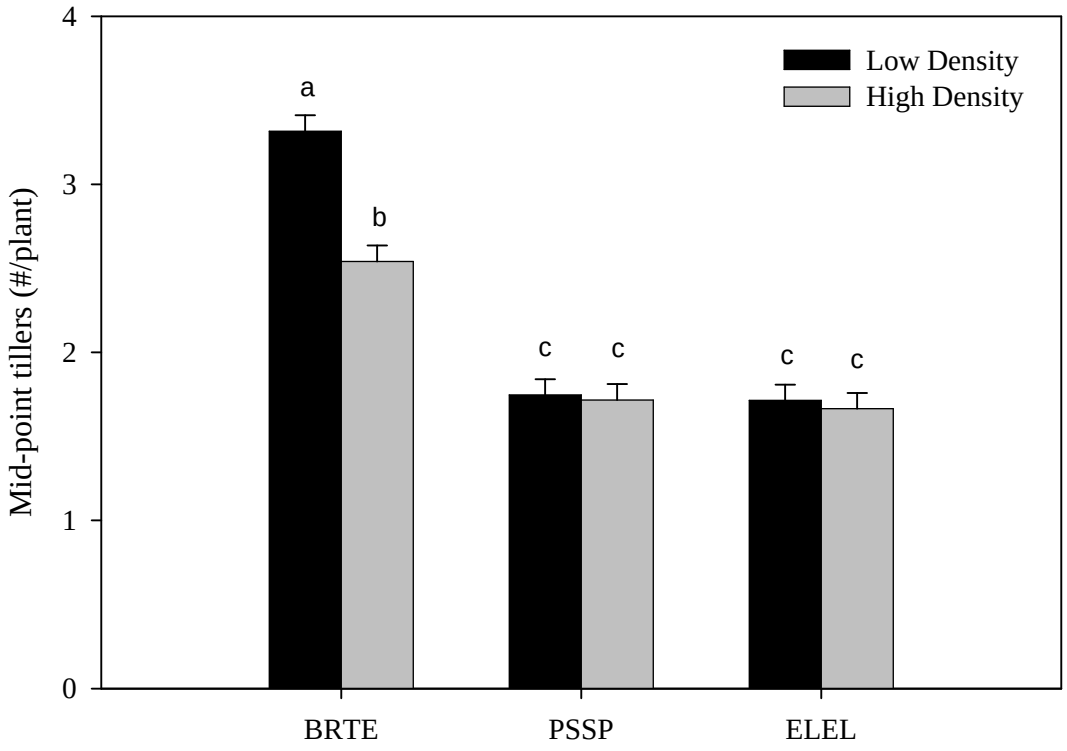

Figure 2.9 The effect of density on mid-point tiller number of *B. tectorum* (BRTE), *P. spicatum* (PSSP), *E. elymoides* (ELEL). Bars represent means of four replicates with error bars representing + 1 standard error. Tiller number values are for 25 days after the water treatments began. Significant differences among treatments are indicated by different letters ($P < 0.01$).

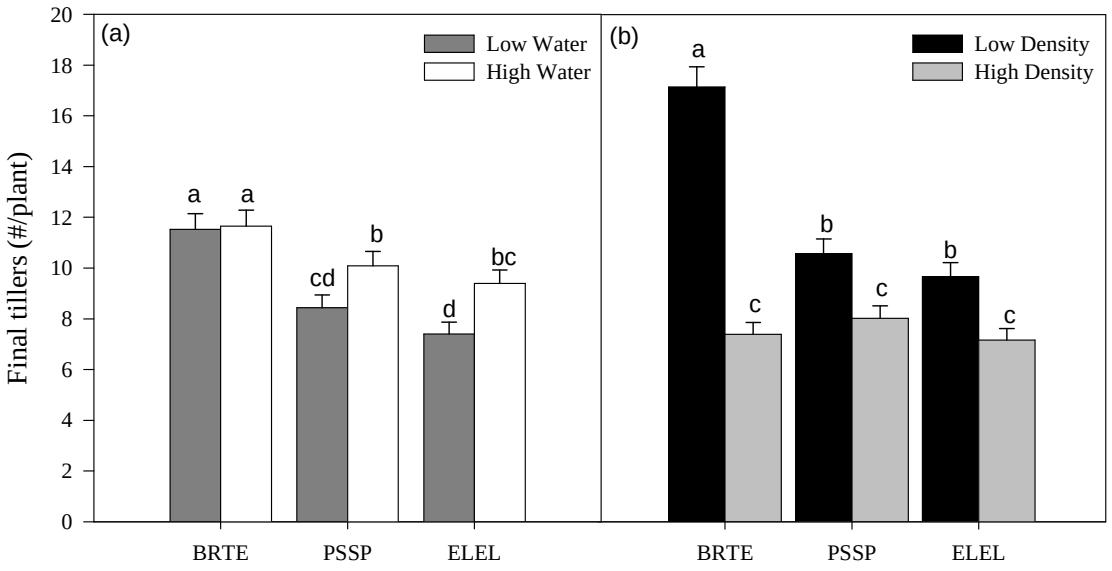

Figure 2.10 The effect of (a) water, and (b) density on final tiller number of *B. tectorum* (BRTE), *P. spicatum* (PSSP), and *E. elymoides* (ELEL). Bars represent means for four replicates with error bars representing + 1 standard error. Final tiller number values were at harvest; 50 days after the water treatments began. Significant differences among treatments are indicated by different letters ($P < 0.01$).

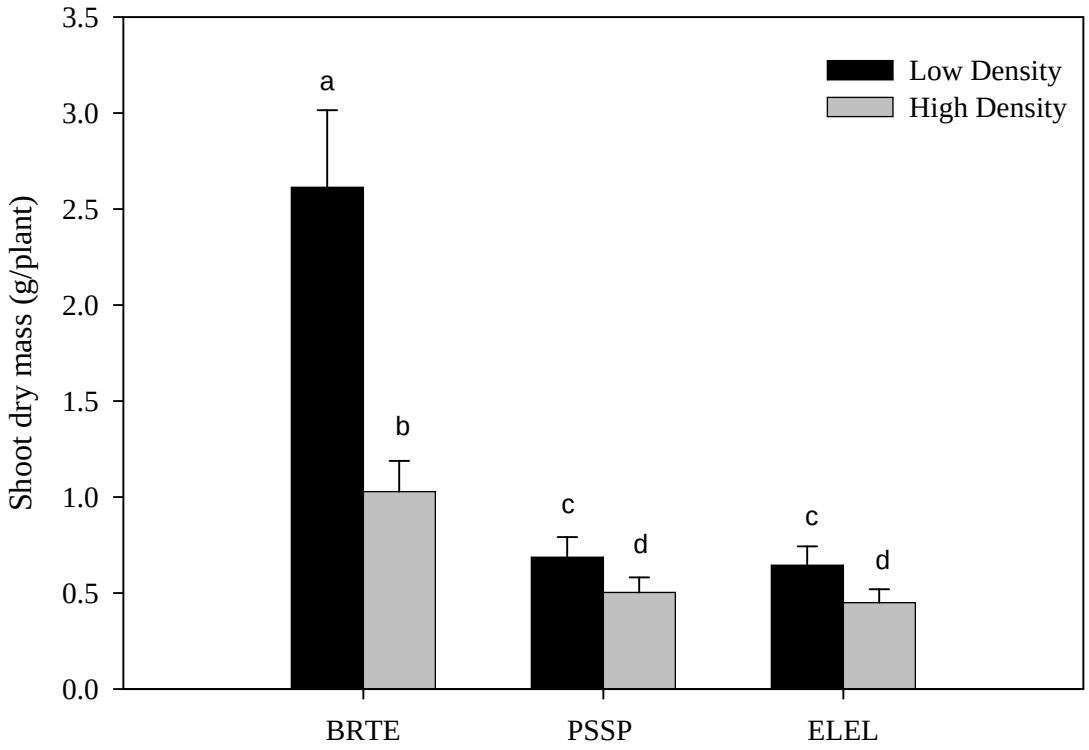

Figure 2.11 The effect of density on shoot dry mass of *B. tectorum* (BRTE), *P. spicatum* (PSSP), and *E. elymoides* (ELEL). Bars represent means of four replicates with error bars representing + 1 standard error. Significant differences among treatments are indicated by different letters ($P < 0.01$).

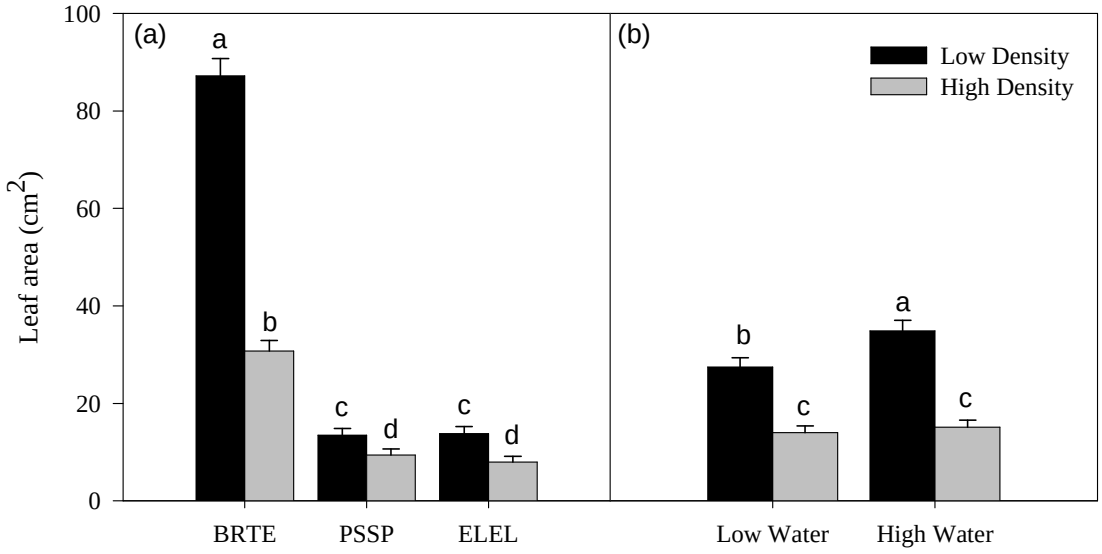

Figure 2.12 The effect of (a) density on leaf area of *B. tectorum* (BRTE), *P. spicatum* (PSSP), and *E. elymoides* (ELEL), and (b) water, and density on leaf area. Bars represent means of four replicates with error bars representing + 1 standard error. Significant differences among treatments are indicated by different letters ($P < 0.01$).

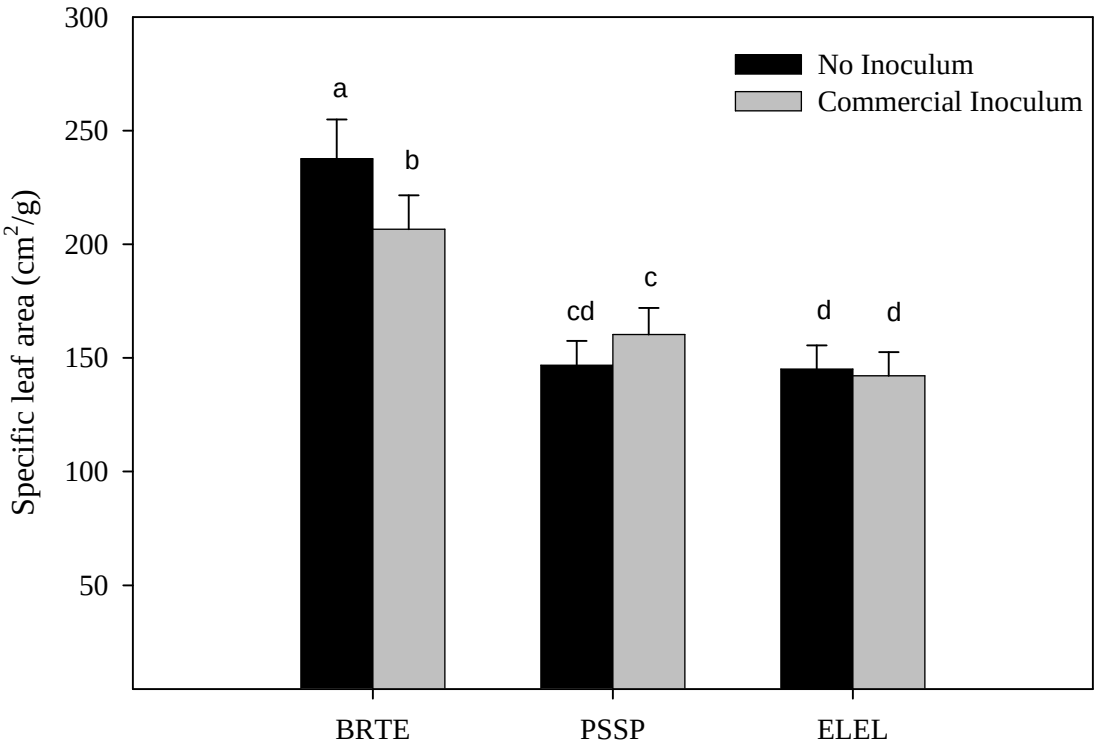

Figure 2.13 The effect of inoculum on specific leaf area of *B. tectorum* (BRTE), *P. spicatum* (PSSP), and *E. elymoides* (ELEL). Specific leaf area is the area of leaf per gram of plant. Bars represent means of four replicates with error bars representing + 1 standard error. Significant differences among treatments are indicated by different letters ($P < 0.01$).

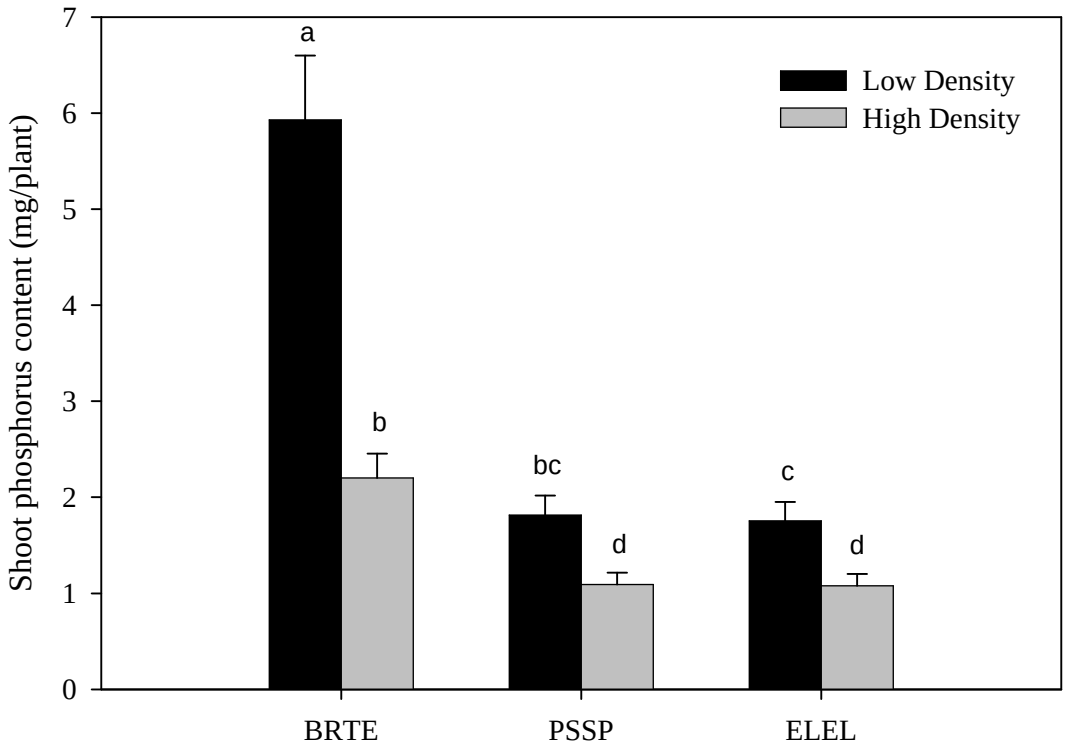

Figure 2.14 The effect of density on shoot phosphorus content of *B. tectorum* (BRTE), *P. spicatum* (PSSP), and *E. elymoides* (ELEL). Bars represent means of four replicates with error bars representing + 1 standard error. Significant differences among treatments are indicated by different letters ($P < 0.01$).

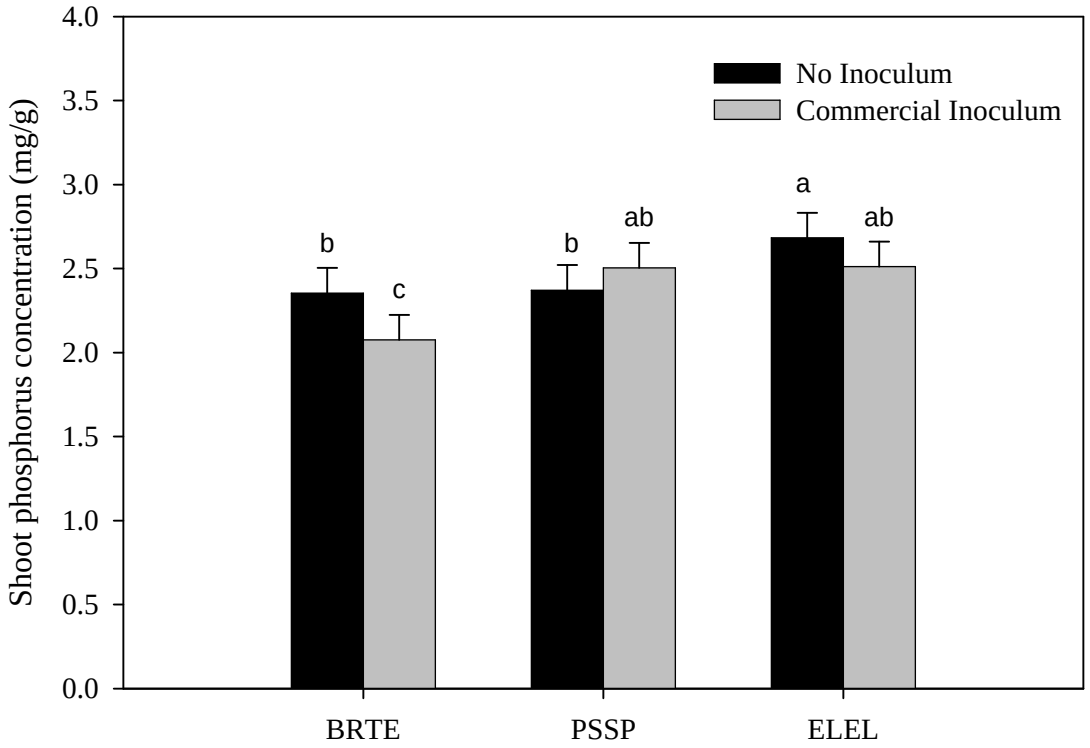

Figure 2.15 The effect of inoculum type on shoot phosphorus concentration of *B. tectorum* (BRTE), *P. spicatum* (PSSP), and *E. elymoides* (ELEL). Phosphorus concentration is milligrams of P per gram of shoot. Bars represent means of four replicates with error bars representing + 1 standard error. Significant differences among treatments are indicated by different letters ($P < 0.01$).

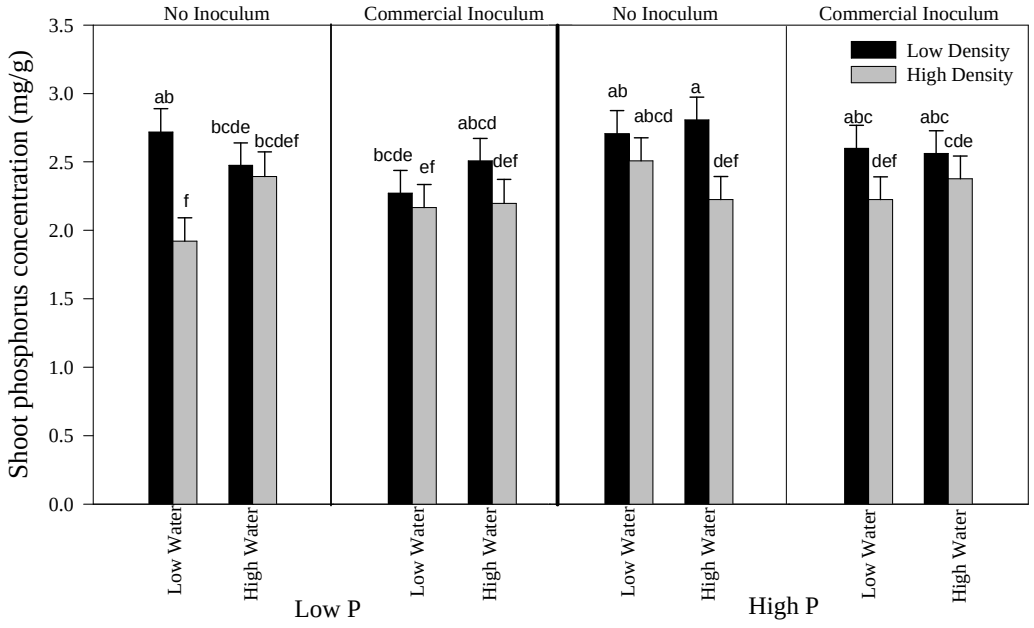

Figure 2.16 The effect of water, inoculum, P, and density on shoot phosphorus concentration across all 3 grass species. Phosphorus concentration is milligrams of P per gram of shoot. Bars represent means of four replicates with error bars representing + 1 standard error. Significant differences among treatments are indicated by different letters ($P < 0.01$).

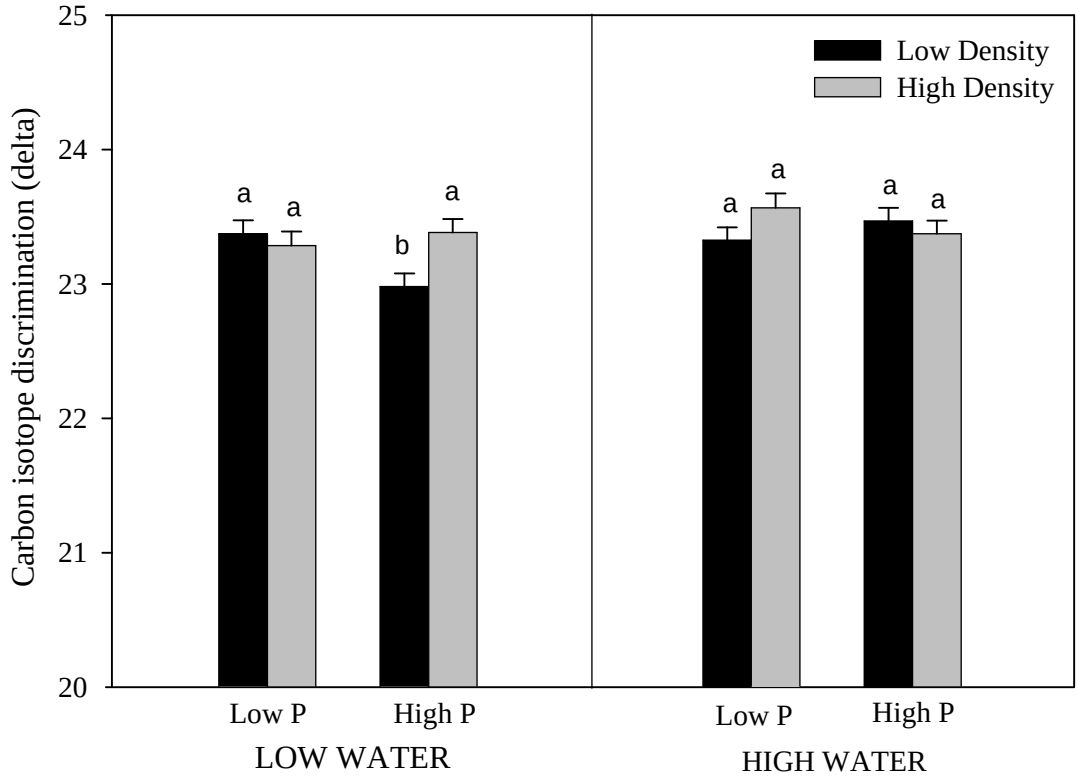

Figure 2.17 The effect of water, P, and density on carbon isotope discrimination across all three grass species. Bars represent means for four replicates with error bars representing + 1 standard error. Carbon isotope discrimination is a measurement of a plant's ability to select against the heavier ^{13}C versus the lighter ^{12}C; the greater the delta, the greater the discrimination suggesting less water stress. Significant differences among treatments are indicated by different letters ($P < 0.01$).

CHAPTER 3

INVASIVE ANNUAL AND NATIVE PERENNIAL COMPETITION MEDIATED BY COMMERCIAL AND LOCAL INOCULA

Summary

The introduction of *Bromus tectorum* has led to highly disturbed systems in the Great Basin, USA, resulting in alterations in both plant and fungal communities. While sowing desirable seeds is common practice post-disturbance, inoculation with arbuscular mycorrhizal fungi may also be important. In this study I investigated the responses of three grasses, the exotic annual *B. tectorum* and the native perennials *Pseudoroegneria spicatum* and *Elymus elymoides*, to commercial inoculum and local inoculum while grown with both conspecifics and heterospecifics. While both inocula generally benefited all three species, the local inoculum tended to have a greater benefit, especially for *B. tectorum*. However, *P. spicatum* shoot dry mass (SDM) response to *B. tectorum*, the *E. elymoide*s SDM response to *P. spicatum* and the whole pot RDM of the *P. spicatum* and *E. elymoides* mixture suggest that during interspecific competition the commercial inoculum may be more beneficial than local inoculum to the perennials.

Introduction

Bromus tectorum and Great Basin plant and arbuscular mycorrhizal fungal communities

B. tectorum invasion has had a severe negative impact on perennial plant communities. In particular it has increased fire frequency in the Great Basin to the detriment of native perennials, leading to a *B. tectorum*-fire cycle (Stewart & Hull, 1949;

Wright, 1985; Knapp, 1996; Humphrey & Schupp, 2004). In addition to the plant community, the arbuscular mycorrhizal fungal community may also be impacted by *B. tectorum* invasion (Hawkes *et al.*, 2006). Alterations to the arbuscular mycorrhizal fungal community could be detrimental to the native plant community through plant-fungus feedbacks (Bever, 1999). Thus, in addition to seeding, inoculum addition may be needed in restoration of *B. tectorum*-invaded systems. Before introducing inoculum to a site it is important first to understand how mycorrhizae mediate competition between *B. tectorum* and native perennials. Then, if inoculum is introduced to a site, it is important to determine the appropriate inoculum source.

Mycorrhizae and plant competition

While mycorrhizal relationships of native perennial grasses often depend on both abiotic and biotic conditions, the mycorrhizal relationship of *B. tectorum* may depend largely on competitor identity, although the exact relationship is not completely clear. *B. tectorum* appears to be mycorrhizal unless grown only with non-mycorrhizal species (Pendleton & Smith, 1983; Reeves *et al.*, 1979). Although *B. tectorum* can be colonized by AMF, the mycorrhizae might not be beneficial and in some cases might even be detrimental (Allen 1984, 1988; Benjamin & Allen, 1987). However, it has also been suggested that AMF might disproportionately enhance the competitive effect of *B. tectorum* on natives (Schwab & Loomis, 1987), or the presence of AMF might benefit both native perennials and *B. tectorum* (Goodwin, 1992). In addition mycorrhizae can in some cases ameloriate competitive effects of invasive annuals on native perennial grasses (Allen & Allen, 1984; Benjamin & Allen, 1987). These studies suggest that the role of

mycorrhizae in competition between *B. tectorum* and native perennials may vary greatly from plant community to plant community and even among sites.

Indigenous versus non-indigenous mycorrhizae and plant restoration

There are many sources of inoculum for restoration practices. These sources include soil from a similar, undisturbed site, an undisturbed site with a different plant community, or commercial inoculum often obtained from a variety of sites. Researchers have found mixed results as to whether indigenous or non-indigenous mycorrhizae best enhance plant recovery.

In the restoration of a weed-infested roadbed, plots inoculated with a commercial AMF blend had less total plant cover and biomass than did plots inoculated with native AMF (DePrenger-Levin *et al.*, 2004). However, the study did not differentiate between native and non-native plant cover and biomass. *B. tectorum* was abundant on all plots, but the dominant species on the native inoculated plots was *Bouteloua gracilis*, whereas the dominant species on the commercial inoculated plots was *B. tectorum*. Similarly, fungal isolates from prairie soil produced a greater response in prairie plant species than did introduced fungal isolates (Hetrick *et al.*, 1986).

The presumed benefit of local AMF is that they likely have adapted to the environmental conditions of a particular site (Lambert *et al.*, 1980). However, if a site's characteristics have been drastically altered, the indigenous fungi may no longer be adapted, and introduced mycorrhizal fungi may benefit the plants more (Powell, 1976, 1977).

The varying effects of indigenous and non-indigenous AMF at different locations

is illustrated in several studies. *Ipomoea batatas* inoculated with AMF from *I. batatas* fields had decreased biomass production compared to plants inoculated with introduced AMF and non-inoculated plants (Hung *et al.*, 1990). However, in a study by Abbott *et al.* (1983), introduced inoculant only benefited clover when the indigenous AMF were ineffective at rapidly and extensively colonizing the roots. Similarly, exotic AMF initially benefited a leguminous shrub, but were not able to maintain their inoculum potential in the field (Requena *et al.*, 2001). In the long term, the indigenous AMF benefited both the leguminous shrub and non-inoculated plants more by improving physiochemical and biological soil conditions and plant health (Carrillo-Garcia. *et al.*, 1999). Further, the effect of the AMF not only depends on the site conditions, but also on plant species and the plant's developmental stage (Ferrol *et al.*, 2004). Thus, AMF mixtures may be more beneficial for restoration than using single species (van der Heijden *et al.*, 1998).

The present study sought to determine the role of mycorrhizae in altering the competitive balance between *Pseudoroegneria spicatum*, *Elymus elymoides*, and *B. tectorum*, and whether commercial inocula and locally cultured inocula are equivalent in their effects. In particular, I addressed three questions: (1) What is the effect of inoculum cultured from a local sagebrush site compared to that of a commercial Basin and High Plains Suite inoculum blend on root dry mass (RDM), root: shoot ratio, shoot dry mass (SDM), specific leaf area (SLA), leaf area, tiller number, WUE, water use, and shoot tissue phosphorus (P) concentration and content, when *P. spicatum*, *E. elymoides*, and *B. tectorum* are grown in monocultures versus when grown with either one or both of the other grass species? (2) To what extent are these results affected by total plant

density? (3) What is the effect of inoculum cultured from a local sagebrush site compared to a commercial Great Basin inoculum blend on % root colonization, root length, and specific root length, when *P. spicatum*, *E. elymoides*, and *B. tectorum* are grown in monoculture at low versus high total plant density.

Methods

Experimental design

I used a 7 x 3 x 2 factorial pot experiment with four replicates in a complete random block design in the greenhouse. Factors were: (1) species with seven levels (*P. spicatum*, *E. elymoides*, and *B. tectorum* in monoculture and in all two- and three-species combinations), (2) inoculum with three levels (no mycorrhizae, or 'no inoculum', locally cultured mycorrhizae, or 'local inoculum', and 'AM120 Basin and High Plains Suite', or 'commercial inoculum'), and (3) density with two levels (6 and 18 total plants per pot). Pots with more than one species had the same number of each species; e.g. high density pots (18 plants per pot) with three species had six plants of each species, while high density pots with two species had nine plants of each species. Species were planted in a circular, regularly spaced pattern. In mixtures, all plants had heterospecific neighbors, and in three-species mixtures each individual had a different heterospecific on either side. The high density pots had an inner circle of six plants and an outer circle of 12 plants. The low density pots had a circle of six plants equivalent in spacing to the inner circle of plants in the high density pots.

Each replicate served as a block to control for potential temperature/humidity gradients in the greenhouse. Each block had a 6 x 8 pot arrangement. Given the space

available, this layout minimized edge effects while allowing all pots to fit on the greenhouse benches.

Study species

The native grasses *P. spicatum* and *E. elymoides* were selected because of their different life history traits and abilities to compete with *B. tectorum*, and because both are desirable native restoration species. *E. elymoides* is a short-lived, early seral perennial that can compete with *B. tectorum* (Hironaka & Tisdale, 1963; Arredondo *et al.*, 1998; Jones, 1998; Booth *et al.*, 2003; Humphrey & Schupp 2004). *P. spicatum* is a long-lived perennial that is not as competitive with *B. tectorum* (Aguirre & Johnson, 1991). Thus, these two species represent two different successional stages allowing for a broader study of the effect of mycorrhizae on invasive and native species in the Great Basin.

Inoculum production

Soil (fine-loamy, mixed, superactive, mesic Xeric Haplocalcid) was obtained from the Onaqui site (0375362N, 4450797E) within the sagebrush-cheatgrass network of the Sagebrush Steppe Treatment Evaluation Project (SageSTEP) in Tooele County, UT, USA, at approximately 1690m elevation. This site was selected because both *E. elymoides* and *P. spicatum* were present and *B. tectorum* had minimal plant establishment. It was also considered the healthiest sagebrush site in the Salt Lake BLM district as determined by the SageSTEP project, so the diversity of AMF species is assumed to be high for this experiment. Soil was dug 5-30 cm beneath the soil surface under *E. elymoides*. *P. spicatum* was also present on the site, but in low numbers. In 115 mL cone-tainers, 40 mL of the soil was layered on 40 mL of terra green and capped with

20 mL of terra green. *Allium* seeds were planted in the cone-tainers. Cone-tainers were watered 1-2 times a day until seedlings were established. Thereafter, pots were watered every 3 days with 7 mL of a 1/50 dilution of a modified Hoagland's solution containing only macronutrients (Feldman & Idczak, 1992). After 8 weeks, the presence of arbuscules and vesicles were found in the onion roots, which were cut into ~1 cm pieces and hand-mixed back into the terra green/soil mixture that they grew in. This terra green-soil-onion root mixture was the local inoculum. The terra green was the same substrate used in the commercial inoculum.

The most probable number (MPN) method was used to assess the infectivity of the final substrate (Daniels & Skipper, 1982) using clover plants. Infectivity levels of the local and commercial inocula were compared. Commercial inoculum and terra green were donated by Reforestation Technologies International, Salinas, CA, USA.

Pot preparation

Due to the cost and time required to collect soil from a local sagebrush site, 6.6 liter pots (22 cm diameter x 21.5 cm height) were filled with a steam-sterilized 1:3 beach sand and topsoil (sandy loam) mixture and mixed with a cement mixer. Sand and topsoil were purchased from Logan Landscape Products, Logan, UT, USA. Soil was sifted with a 2-mm sieve. A 1:3 beach sand and topsoil mixture was used because it had a P of 8.8 mg/kg of soil. A low P level was desired because in a previous experiment (Chapter 2), AMF had a detrimental effect when the soil P was 20 mg/kg.

A layer of 450 mL of commercial or local inoculum or sterilized terra green (substrate of inoculum, 'the control') was added to each pot and capped with soil to help prevent cross contamination. In order to ensure root contact with the inoculum and

reduce the amount of inoculum needed, the inoculum was layered rather than mixed throughout the soil.

P. spicatum (Anatone) and *E. elymoides* seeds were obtained from the U.S. Department of Agriculture Agricultural Research Service Forage & Range Research Laboratory, Logan, UT, USA. *B. tectorum* seeds were collected from Simpson Springs and Vernon Hills, Tooele County, Utah, USA. Three seeds for every one desired individual were planted directly into the pots (no pre-germination), and any extra seedlings were thinned immediately on emergence. As a back up, seeds were pre-germinated in germination boxes for 1-2 weeks using tetramethyl-thiuram disulfide (fungicide); these seedlings were used to replace the few missing seedlings or seeds that did not germinate within 3 weeks of planting. Replacement seedlings were approximately the same size as the seedlings in the pots and were planted before any roots in the pot had reached the inoculum layer – that is before any treatments began. Plants that died later in the experiment were attributed to treatment effect and were not replaced. Since *B. tectorum* has a faster germination rate, it was planted 1 week after the perennials so that the species were all initially approximately the same size when water stress began. Pots were watered with a mister for 17 days after *B. tectorum* seeds were planted to ensure establishment. Thereafter, the water stress phase of the experiment began. Plants were harvested 44 days after water stress began.

Watering regime

A WP4-T- Dewpoint PoteniaMeter (Decagon Inc. Pellman, WA) was used to determine that 2% soil water content occurred at – 1.5 MPa. Once the water stress phase of the experiment began, each pot was initially watered when it reached 2.5-3.5% soil

water content. Plants began showing signs of severe stress 20 days into this phase, so the watering regime was adjusted so that each pot was watered when its soil water content reached 7-9.5%. When watered, all pots were brought up to field capacity, 15% water content.

Six WUE control pots were added to each replicate. These control pots were filled with soil (equivalent weight to other pots), but did not contain plants. The control pots were used to account for evaporation of water from the soil in water use and WUE calculations.

MPN method

A 1:1 mixture of native soil (source of local inoculum) and sand was autoclaved for 55 minutes with an additional 20-minute exhaust period. It was cooled and dried overnight. A 10-fold series dilution up to 10^{-5} with 5 replicates was mixed as follows for both commercial and local inoculum: For 10^{-1}, 50g of inoculum was thoroughly mixed with 450 g of sterilized soil (1:1 native soil: sand) in a Ziploc bag by shaking it 100 times (Porter, 1979). For 10^{-2}, 50g of 10^{-1} diluted inoculum was thoroughly mixed with 450 g of sterilized soil as described above. This was repeated up to 10^{-5}.

Clover seeds were planted in 115 mL conetainers with each dilution's soil. Five control conetainers containing only the sterilized soil and sand mixture and clover plants were used to ensure that the soil was not infected by mycorrhizae. After 8 weeks, the clover roots were washed and stored in 50% ethanol.

Physiological and morphological measurements

Measured responses to mycorrhizae were: percent mycorrhizal root colonization, SRL, leaf area, SLA, root length, tiller number, RDM, SDM, root:shoot ratio, shoot P content and concentration, water use, and WUE. Due to the short time frame of the experiment, plants were harvested and responses measured only at the end of the experiment.

A belt-driven leaf area meter was used to measure leaf area on freshly harvested shoots. To measure root length in monospecific pots, the roots were lightly washed, floated in transparent trays containing water, scanned with a flatbed scanner at 300 dpi, and analyzed using an image analysis program (WinRhizo, Regent Instruments Inc., Quebec City, Canada). To determine dry mass, shoots and roots were oven dried at 70˚ C for 7 and 3 days, respectively, and weighed.

While harvesting each root mass, a 1-2 g root sample for mycorrhizal quantification was cut and stored in 50% ethanol. Each sample had four subsamples, two from shallower and two from deeper roots. The dry weight of the samples used for mycorrhizal quantification was estimated and added to the total root weight using each mycorrhizal root sample's fresh weight and the corresponding root mass' fresh/dried weight. Measurements of mycorrhizal colonization are described in the 'staining for mycorrhizae' section below.

The effect of mycorrhizae on P uptake was assessed by comparing shoot P concentration and P content between the treatments. Ground tissue samples were analyzed by the Soil and Plant Analysis Laboratory at Brigham Young University, Provo,

UT, USA using the nitrate perchlorate method. Inductively coupled plasma (ICP) was used to analyze the extractions.

To analyze differences in water use efficiency between treatment combinations, root water use efficiency (root dry mass/water use), shoot water use efficiency (shoot dry mass/water use) and total water use efficiency (root+shoot dry mass/water use) were all calculated.

Shoot tissue P content, root length, leaf area, and tiller number means are per plant values. RDM, root:shoot ratios, water use, shoot WUE, root WUE, and WUE are whole pot values. Roots were not separated by species due to the difficulty in properly identifying species' roots. SDM is reported both as whole pot and per plant values so that comparisons could be made between growth, water use and water use efficiency. All references to the SDM of particular species are per plant values unless otherwise specified.

Staining for mycorrhizae

Roots for mycorrhizal quantification and MPN determination were stained using the protocol in Phillips & Hayman (1970), optimized for the type of roots being stained and to reduce the use of toxic chemicals. Roots were cleared for 30 minutes and stained for 12 minutes. Lactoglycerol rather than lactophenol was used in the 0.05% trypan blue staining solution and to store stained root specimen.

For monospecific pots, hyphal, arbuscular and vesicular colonization was measured using the magnified gridline intersect method and a 400x magnification lens (Giovannetti & Mosse, 1980; McGonigle *et al.*, 1990). For heterospecific pots, presence

or absence of mycorrhizae was determined by placing 10, ~1 cm root segments on a slide and examining the entire length of each root at 400x magnification.

For MPN determination, roots were examined under 400x magnification until mycorrhizal structures were observed or the entire root system had been examined. MPN was determined using the table in Alexander (1965).

Statistical analyses

All statistical analyses were performed using SAS v 9.1.3 (2003). A mixed model 3-way ANOVA was used to determine the effect of each fixed factor combination on root:shoot ratios, whole pot SDM, whole pot RDM, per plant root length, SRL, shoot WUE, root WUE, WUE and whole pot water use. The three fixed, explanatory factors were species, density, and inoculum. For per plant root length and SRL only monocultures were analyzed. For root:shoot ratios, whole pot SDM, RDM, shoot WUE, root WUE, total WUE, and water use, the measurement unit was the pot. For shoot tissue P content, shoot tissue P concentrations, SLA, root length, leaf area, tiller number and SDM, the measurement unit was each species within the pot.

A 4-way ANOVA was used to determine the effect of each fixed factor combination on the following 18 response variables: tiller number, P content, P concentration, leaf area, SLA and SDM (n = 6) for each species (n = 3). The four fixed, explanatory factors were presence/absence of species A, presence/absence of species B, density and inoculum. For example, for *P. spicatum* SDM, the explanatory factors were presence/absence of *B. tectorum*, presence/absence of *E. elymoides*, density and inoculum.

Contingency table analyses using the chi-square test showed that both commercial inoculum and local inoculum pots differed from the no inoculum pots in the presence/absence of mycorrhizae; i.e., the non-inoculated pots were truly control pots. A 3-way ANOVA using species, density, and inoculum as explanatory factors was performed to determine the effects of treatment combinations on percent colonization of inoculated monospecific pots. For the inoculum explanatory factor, only two levels were used: commercial inoculum and local inoculum. Correlational analyses were conducted using the PROC CORR command in SAS v 9.1.3 (2003) in order to determine patterns among per plant SDM and P concentration. Confidence intervals for MPN were calculated using the tables in Alexander (1965). For all other measurements, least squares means and standard errors were calculated.

Statistical significance was set at the 0.01 probability level. This level was used because many higher order interactions were significant at the 0.05 level, but the component lower order interactions and/or main effects were not, making their significance suspect. In addition, because many independent analyses were performed, the 0.01 level helps control for an inflated probability of finding a significant difference without being constrained by the extremely conservative Bonferroni correction. Lastly, the 0.01 level provides higher confidence for extrapolation of data into field situations.

The following response variables were transformed as indicated to meet assumptions of normality and homogeneity of variance. *P. spicatum* P concentration, root:shoot ratios, root length, *B. tectorum* P content, *B. tectorum* leaf area and *P. spicatum* leaf area were square root-transformed. Percent root colonization, root WUE, *E. elymoides* SDM, *B. tectorum* tiller number *P. spicatum* tiller number and *E. elymoides*

leaf area were cube root-transformed. *P. spicatum* SDM, SRL, shoot WUE, *E. elymoides* tiller number and *P. spicatum* P content were log-transformed. *B. tectorum* SDM, *E. elymoides* SLA and *E. elymoides* P content were quarter-root transformed**.** Least squared mean comparisons were made for all statistically significant interactions and/or main effects. All least squared means and standard errors were back-transformed for figures, tables and text.

Results

Mycorrhizal colonization and MPN

The contingency analyses showed that the presence of mycorrhizae in commercial inoculum pots (56 present/0 absent) and in local inoculum pots (47/9) differed significantly from presence in no-inoculum pots (6/50) (χ^2=90.3226; df=1; P <0.0001 and χ^2=60.2085; df=1; P <0.0001; respectively). Three of the six non-inoculated pots that contained colonized roots monocultures, which were quantified for percent root colonization; had $\leq$ 10% colonization. The other three pots did not have high levels of colonization. These six pots were analyzed as no inoculum pots because colonization levels were low and the source of mycorrhizae was not known. The nine pots inoculated with local inoculum that had no root colonization were kept in the analysis because even though colonization was not detected, the local inoculum may still have affected the physiology and morphology of the plants.

Percent root colonization was significantly affected by species, inoculum, and the species x inoculum interaction (Table 3.1). Overall, *B. tectorum* had significantly less colonization than *P. spicatum*; no other species comparison differed (*B. tectorum*: 11.56% $\pm$ 3.23 *P. spicatum*: 33.74% $\pm$ 6.44 *E. elymoides*: 24.78% $\pm$ 5.28). Commercial

inoculum had greater root colonization than local inoculum, but this was only significant for *B. tectorum* roots, which explains the significant species x inoculum interaction (Figure 3.1).

Commercial inoculum had a greater infectivity than local inoculum, but the difference was not significant due to extremely large confidence intervals. Commercial inoculum had 14,000 propagules/50g of inoculum with a confidence interval of 4,242-46,200. The local inoculum had 4,300 propagules/50g of inoculum with a confidence interval of 1,303-14,190.

Root dry mass

Whole pot root dry mass was significantly influenced by species, density, inoculum, and the species x inoculum and density x inoculum interactions (Table 3.1). Whole pot RDM of the *E. elymoides* monocultures (1.61 g $\pm$ 0.27) was significantly less than RDM of *P. spicatum* monocultures (2.42 g $\pm$ 0.27), *B. tectorum-P. spicatum* mixtures (2.47 g $\pm$ 0.27), *E. elymoides-B. tectorum* mixtures (2.37 g $\pm$ 0.27), and the three-species mixtures (2.44 g $\pm$ 0.27). Whole pot RDM did not significantly differ for any other species combination.

The high density treatment had significantly greater whole pot RDM than the low density treatment (Figure 3.2). Plants grown in the local inoculum treatment (2.81g $\pm$ 0.24) had significantly greater whole pot RDM than plants in the commercial inoculum treatment (2.29 g $\pm$ 0.24), which had significant greater whole pot RDM than the no-inoculum treatment (1.56 g $\pm$ 0.24).

In monoculture treatments, *B. tectorum* whole pot RDM was significantly greater in the local inoculum than in the other two treatments which did not differ, *P. spicatum*

whole pot RDM did not differ between local and commercial inoculum treatments, both of which were significantly greater than the no-inoculum treatment, and *E. elymoides* whole pot RDM did not differ between treatments (Figure 3.3). The *B. tectorum-P. spicatum* mixture had significantly greater whole pot RDM in the local inoculum treatment than in the no-inoculum treatment, while the intermediate commercial inoculum treatment did not differ significantly from either of the other two treatments. The *E. elymoides-B. tectorum* mixture had significantly greater whole pot RDM in the local inoculum treatment than in the commercial and no-inoculum treatments, which did not differ from each other. The *E. elymoides-P. spicatum* mixture had statistically equivalent whole pot RDM between all three inoculum treatments. Whole pot RDM in the *B. tectorum-P. spicatum-E. elymoides* mixture was significantly greater in the local inoculum treatment than in the commercial inoculum treatment, which was significantly greater than in the no-inoculum treatment (Figure 3.3). The differing responses of the seven species combinations to the inoculum treatments explain the significant species x inoculum interaction.

The density x inoculum interaction shows that while plants within all three inoculum treatments had significantly greater whole pot RDM when plant density was greater, the local inoculum treatment had the greatest response to density (Figure 3.2). Whole pot RDM of high density, no-inoculum pots was similar to that of low density pots inoculated with either inoculum. At high density, local inoculum pots had significantly greater whole pot RDM than commercial inoculum, and commercial inoculum had significantly greater whole pot RDM than the no-inoculum pots (Figure 3.2).

SRL and root length

Specific root length was significantly influenced by species, inoculum, and the species x inoculum interaction (Table 3.1). *B. tectorum* had greater SRL than *E. elymoides* which had greater SRL than *P. spicatum* (Figure 3.4). Overall, the no-inoculum treatment had significantly greater SRL than the commercial and local inoculum treatment, which did not differ (Figure 3.4). However, *B. tectorum* SRL did not respond to inoculation while both perennials had an equivalent decrease in SRL with both commercial and local inoculum relative to no inoculum, which can explain the significant species x inoculum interaction (Figure 3.4).

Root length per plant for monocultures was significantly affected by species, density, inoculum, and the species x density and species x inoculum interactions (Table 3.1). *B. tectorum* had significantly greater per plant root length than *P. spicatum* and *E. elymoides*, which had similar per plant root lengths (Figure 3.5). All three species had reduced per plant root length in the high density treatment relative to the low density treatment (Figure 3.5). However, the response was not significant for *P. spicatum*, intermediate for *E. elymoides* and greatest for *B. tectorum*, which explains the significant species x density interaction. The species x inoculum interaction arises because both *P. spicatum* and *E. elymoides* per plant root lengths did not differ between inoculum treatments while *B. tectorum* per plant root length significantly increased in the local inoculum treatment compared to the two other inoculum treatments (Figure 3.6).

Total WUE, shoot WUE, and root WUE

The species main effect was the only significant parameter for total WUE (Table 3.1). *B. tectorum* monocultures had significantly greater total WUE than both perennial monocultures and the perennial species mixture (Table 3.2). However, all mixtures which included *B. tectorum* did not differ from the *B. tectorum* monoculture. The perennial mixture had the lowest total WUE, although it was only significantly less than the *B. tectorum* monoculture and the three-species mixture (Table 3.2).

The species main effect was also the only significant parameter for shoot WUE (Table 3.1). *B. tectorum* monocultures had significantly greater shoot WUE than both perennial monocultures, the perennial mixture and the *B. tectorum-P. spicatum* mixture. All other treatment combinations were statistically equivalent (Table 3.2).

In contrast to total WUE and shoot WUE, root WUE was significantly affected by density and inoculum (Table 3.1). The no-inoculum and commercial inoculum treatments had significantly greater root WUE (9.88E-04 $\pm$ 9.99E-05 and 9.51E-04 $\pm$ 9.78E-05) than the local inoculum treatment (8.13E-04 $\pm$ 8.76E-05). Root WUE was significantly greater in the high density treatment (7.93E-04 $\pm$8.42E-05) than in the low density treatment (1.05E-03$\pm$1.00E-04).

Total water use

Total water use was significantly affected by species, density, inoculum, and the species x inoculum interaction (Table 3.1). Total water use of *B. tectorum* monocultures (2681.38 g $\pm$ 148.41) was significantly greater than total water use of *P. spicatum* monocultures (2101.17 g $\pm$ 148.41) and *E. elymoides* monocultures (1745.96 g $\pm$ 148.41). Total water use of *E. elymoides* monocultures was significantly less than total water use

in all mixtures containing *B. tectorum* (*P. spicatum-B. tectorum*: 2629.29 g ± 148.41, *E. elymoides-B. tectorum*: 2623.04 g ± 148.41, three-species mixture: 2456.38 g ± 148.41). No other species combinations differed significantly.

Total water use was significantly greater in the high density treatment (2676.61 g/pot ± 86.54) than in the low density treatment (2027.50 g/pot ± 86.54). Local inoculum treatment total water use (3191.49 g ± 101.96) was significantly greater than that of commercial inoculum treatment (2216.05 g ± 101.96), which was significantly greater than that of no-inoculum treatment (1648.64 g ± 101.96).

B. tectorum monocultures, *P. spicatum-B. tectorum* mixtures and *E. elymoides-B. tectorum* mixtures had significantly greater total water use in the local inoculum treatment than in the commercial and no-inoculum treatments, which did not differ from each other (Figure 3.7). *P. spicatum* monocultures had significantly greater total water use in the local inoculum treatment than in the no-inoculum treatment; the commercial inoculum treatment was intermediate and did not differ from either of the other two inoculum treatments. *E. elymoides* monocultures had significantly greater total water use in the local inoculum treatment than in the commercial treatment; the no-inoculum treatment was intermediate and did not differ from either of the other inoculum treatments (Figure 3.7). *P. spicatum-E. elymoides* mixtures had significantly greater total water use in the commercial inoculum treatment than in the no-inoculum treatment; the local inoculum treatment was intermediate and did not differ from either of the other treatments. Lastly, *B. tectorum-P. spicatum-E. elymoides* mixtures had significantly greater total water use in the local inoculum treatment than in commercial inoculum treatment which was significantly greater than in the no-inoculum treatment (Figure 3.7).

The differing responses of the seven species combinations to the inoculum treatments explain the significant species x inoculum interaction. Interestingly, *B. tectorum* had significantly greater total water use than the perennial monocultures and the perennial-only mixture only in the local inoculum treatment relative to the no and commercial inoculum treatments (Figure 3.7).

Root:shoot ratios

Root:shoot ratios were significantly affected by species, density, and inoculum (Table 3.1). *B. tectorum* monocultures had significantly lower root:shoot ratios than all other monocultures and mixtures. Additionally, *P. spicatum* had significantly higher root:shoot ratios than *E. elymoides-B. tectorum* mixtures, and *P. spicatum-E. elymoides-B. tectorum* mixtures. *P. spicatum-E. elymoides* mixtures had significantly higher root:shoot ratios than *E. elymoides-B. tectorum* mixtures. All other treatment combinations had statistically equivalent root:shoot ratios (Table 3.3).

The high density treatment had significantly greater root:shoot ratios than the low density treatment (Table 3.3). The commercial inoculum treatment had significantly greater root:shoot ratios than the no-inoculum treatment which was significantly greater than the local inoculum treatment (Table 3.3).

Shoot dry mass

Whole pot SDM was significantly affected by species, density, inoculum, and the species x inoculum and density x inoculum interactions (Table 3.1).

B. tectorum monoculture (6.55 g $\pm$ 0.37) had significantly greater whole pot SDM than did all other species monocultures and mixtures. Species mixtures containing *B.*

tectorum had similar whole pot SDMs (*P. spicatum-B. tectorum* 5.07 g ± 0.37; *E. elymoides-B. tectorum* 5.48 g ± 0.37; *P. spicatum-E. elymoides-B. tectorum* 5.31 g ± 0.37) that were significantly greater than whole pot SDM in perennial monocultures (*P. spicatum* 3.78 g ± 0.37, *E. elymoides* 3.46 g ± 0.37) and perennial mixtures (3.62 g ± 0.37) whole pot SDMs, which did not differ.

Overall, whole pot SDM was significantly greater in the high density treatment (5.15 g ± 0.3124) than in the low density treatment (4.36 g ± 0.3124). Whole pot SDM in the local inoculum treatment (6.53 g ± 0.32) was significantly greater than in the commercial inoculum treatment (4.31 g ± 0.32), which was significantly greater than in the no inoculum treatment (3.42 g ± 0.32). However, the density x inoculum interaction shows that whole pot SDM significantly differed between low and high density treatments only for the local inoculum treatment. It also shows that at low density all three inoculum treatments differed significantly while at high density the commercial inoculum treatment did not differ from the no-inoculum treatment (Figure 3.8).

The species x inoculum interaction reveals that different species combinations respond differently to the three inoculum treatments. Monocultures of *B. tectorum*, mixtures of *P. spicatum -B. tectorum*, and mixtures of *E. elymoides-B. tectorum* had significantly greater whole pot SDM in the local inoculum treatment than in the commercial and no-inoculum treatments, which did not differ from each other (Figure 3.9). Both *P. spicatum* and *E. elymoides* monocultures had significantly greater whole pot SDM in the local inoculum treatment than in the no-inoculum treatment; the commercial inoculum treatment was intermediate not differing from either the no or the local inoculum treatments. In the perennial mixture, whole pot SDM did not differ

between the local and commercial inoculum treatments but both of these had greater whole pot SDM than the no-inoculum treatment. The three-species mixture had significantly greater whole pot SDM in the local inoculum treatment than in the commercial inoculum treatment, which was significantly greater than in the no-inoculum treatment.

In addition to whole pot SDM, per plant SDM for each species was also measured. *B. tectorum* per plant SDM was significantly affected by the presence/absence of *E. elymoides* (hereafter referred to as ELEL), density, and the ELEL x inoculum interaction (Table 3.4a). *B. tectorum* had significantly greater SDM in the low density treatment (1.19 g $\pm$ 0.13) versus the high density treatment (0.40 g $\pm$ 0.06). *B. tectorum* per plant SDM was significantly greater when *E. elymoides* was present versus absent for both the local and commercial inoculum treatments, but not in the no inoculum treatment which explains the significant ELEL x inoculum interaction (Figure 3.10).

P. spicatum per plant SDM was significantly affected by the presence/absence of *B. tectorum* (hereafter referred to as BRTE), density, inoculum, and the BRTE x inoculum interaction (Table 3.4b). *P. spicatum* per plant SDM was significantly greater in the low density treatment (0.44 g $\pm$ 0.02) than in the high density treatment (0.22 g $\pm$ 0.01). Commercial and local inoculum treatments (0.38 g $\pm$ 0.02 and 0.33 g $\pm$ 0.02 respectively) had significantly greater per plant SDM than the no-inoculum treatment (0.25 g $\pm$0.02). The significant BRTE x inoculum interaction shows that *P. spicatum* per plant SDM was significantly greater when *B. tectorum* was absent versus present in the local and commercial inoculum treatments although the difference was only significant in the local inoculum treatment (Figure 3.11).

E. elymoides per plant SDM was significantly affected by BRTE, density, inoculum, and the BRTE x density, *P. spicatum* (hereafter referred to as PSSP) x inoculum, and PSSP x BRTE x density interactions (Table 3.4c). *E. elymoides* had significantly greater per plant SDM in the local inoculum (0.31 g $\pm$ 0.02) than in the no-inoculum treatment (0.23 g $\pm$ 0.01); commercial inoculum treatment per plant SDM (0.26 g $\pm$ 0.02) was intermediate and did not differ from the other inoculum treatments. The significant PSSP x inoculum interaction shows that the competitive effect of *P. spicatum* on *E. elymoides* per plant SDM depended upon the inoculum treatment. *E. elymoides* per plant SDM was significantly greater when *P. spicatum* was absent versus present in the no inoculum treatment, but not in the other two inoculum treatments (Figure 3.12).

The significant PSSP x BRTE x density interaction shows that the competitive effect of *P. spicatum* on *E. elymoides* per plant SDM also depended upon both the presence/absence of *B. tectorum* and the density treatment. In the low density treatment, the presence of either *B. tectorum* or *P. spicatum* significantly reduced *E. elymoides* per plant SDM compared to when both were absent, but the presence of *B. tectorum* had a significantly greater effect than the presence of *P. spicatum*. When both *B. tectorum* and *P. spicatum* were present, *E. elymoides* per plant SDM was greater than when only *B. tectorum* was present, but lower than when *P. spicatum* was present, but the differences were not significant. In the high density treatment, *E. elymoides* per plant SDM was not significantly affected by the presence of either or both species (Figure 3.13).

Tiller number

B. tectorum per plant tiller number was significantly affected by ELEL, density, inoculum, and the ELEL x inoculum, PSSP x ELEL x density, and PSSP x ELEL x

inoculum interactions (Table 3.4a). *B. tectorum* per plant tiller number was significantly greater in the local inoculum treatment (10.47 ± 1.15) than in the commercial inoculum treatment (7.92 ± 0.98) which was significantly greater than the no-inoculum treatment (5.81 ± 0.78).

B. tectorum per plant tiller number responded to the density treatment in the presence of either or both perennials by producing more tillers in the low density treatment than in the high density treatment, but did not respond to density in monoculture (Figure 3.14). When perennials were present, per plant tiller number was significantly greater in the low density treatment than in the high density treatment with the magnitude of difference between the density treatments being greater in the two-species mixtures versus the three-species mixture; that is, in the high density treatment, the three-species mixture had significantly greater per plant tiller number than the two-species mixtures. When both perennials were absent, tiller numbers did not differ between the two density treatments (Figure 3.14); these patterns among the treatment combinations explain the significant PSSP x ELEL x density interaction.

The significant PSSP x ELEL x inoculum interaction shows that the response of *B. tectorum* response to the inoculum treatments depended on the presence/absence of the perennials. In monoculture, *B. tectorum* per plant tiller number was significantly greater in the local inoculum treatment (9.66 ± 1.65) than in the commercial (5.23 ± 1.11) and no-inoculum treatments (4.79 ± 1.05), which did not differ. When only *E*. elymoides was present, *B. tectorum* per plant tiller number was greater in the commercial inoculum treatment (11.58 ± 1.97) than in the no-inoculum treatment (5.36 ± 1.12); local inoculum per plant tiller number (9.21 ± 1.60) was intermediate and did not differ from the other

inoculum treatments. When only *P. spicatum* was present, *B. tectorum* per plant tiller number did not differ among the inoculum treatments (no-inoculum, 7.71 ± 1.42; commercial inoculum, 6.30 ± 1.33; local inoculum, 7.61 ± 1.41). In the three-species mixture, *B. tectorum* had significantly greater per plant tiller number in the local inoculum treatment (16.70 ± 2.35) than in the commercial inoculum (9.62 ± 1.64) and no-inoculum (5.63 ± 1.16) treatments which did not differ significantly (Figure 3.15).

Both *P. spicatum* and *E. elymoides* per plant tiller number were significantly affected by BRTE and density (Tables 3.4b, c). Both *P. spicatum* and *E. elymoides* per plant tiller numbers were greater when *B. tectorum* was absent (*P. spicatum* 5.72 ± 0.39; *E. elymoides* 5.12 ± 0.26) versus when it was present (*P. spicatum* 4.61 ±0.34; *E. elymoides* 3.99 ± 0.20). Both perennials had significantly greater per plant tiller numbers at low density (*P. spicatum* 6.64 ± 0.44; *E. elymoides* 5.69± 0.28) versus at high density (*P. spicatum* 3.89 ± 0.30; *E. elymoides* 3.59 ± 0.18).

E. elymoides per plant tiller number was significantly greater in the local inoculum treatment (5.39 ± 0.33) than in the no-inoculum treatment (3.85 ± 0.24). The commercial inoculum treatment (4.46 ± 0.27) did not significantly differ from the local inoculum and no-inoculum treatments.

B. tectorum had an 80% increase in tiller number, *E. elymoides* had a 55% increase in tiller number and *P. spicatum* had a nonsignificant 18% increase in tiller number when inoculated with the local inoculum versus not being inoculated.

SLA and leaf area

B. tectorum SLA was significantly affected by inoculum and the PSSP x inoculum interaction (Table 3.4a). The significant PSSP x inoculum interaction shows

that *B. tectorum* increased its SLA when grown with *P. spicatum* relative to without *P. spicatum* in the no-inoculum treatment.

P. spicatum SLA was significantly affected by the inoculum main effect (Table 3.4b). *P. spicatum* had significantly greater SLA in the local inoculum treatment (80.86 $cm^2/g \pm 3.64$) than in the commercial (68.01 $cm^2/g \pm 3.67$) and no inoculum treatments ($69.78 cm^2/g \pm 3.64$), which did not differ.

E. elymoides SLA was significantly affected by the BRTE x density interaction (Table 3.4c). Regardless of whether or not *P. spicatum* was present, when *B. tectorum* was absent, *E. elymoides* SLA did not respond to the density treatment, when *B. tectorum* was present, SLA decreased as density increased (Figure 3.17).

B. tectorum per plant leaf area was significantly affected by ELEL, density, inoculum, and the ELEL x inoculum, and density x inoculum interactions (Table 3.4a). Since PSSP was not a significant main effect or component of an interaction, the presence of *P. spicatum* in the *P. spicatum-B. tectorum* and three-species mixtures did not affect *B. tectorum* per plant leaf area. *B. tectorum* had the greatest per plant leaf area in the local inoculum and the lowest per plant leaf area in the no-inoculum treatment (Figure 3.18); all inoculum treatments differed significantly from one another. The significant ELEL x inoculum interaction shows that *B. tectorum* per plant leaf area did not respond to the presence of *E. elymoides* in the no-inoculum treatment, while it increased per plant leaf area in the presence of *E. elymoides* in the commercial and local inoculum treatments (Figure 3.18). Across all three inoculum treatments, *B. tectorum* had greater per plant leaf area in the low density treatment than the high density treatment; however, the

significant density x inoculum interaction shows that *B. tectorum* had the greatest response to the density treatments in the local inoculum treatment (Figure 3.19).

P. spicatum per plant leaf area was significantly affected by BRTE, density, inoculum, and the BRTE x inoculum interaction (Table 3.4b). *P. spicatum* had significantly greater leaf area in the low density treatment (33.55 $\pm$ 1.46) than in the high density treatment (16.21 $\pm$ 1.00). The significant BRTE x inoculum interaction shows the different response of *P. spicatum* per plant leaf area to inoculation when *B. tectorum* was present versus absent. In the absence of *B. tectorum*, inoculation significantly increased *P. spicatum* per plant leaf area relative to the no inoculum (18.75 cm^2/plant $\pm$ 1.78) treatment; the commercial (30.43 cm^2/plant $\pm$ 2.26) and local inoculum (36.33 cm^2/plant $\pm$ 2.46) treatments did not differ significantly. In contrast, when *B. tectorum* was present, *P. spicatum* per plant leaf area did not differ significantly among the inoculum treatments (none, 17.52 cm^2/plant $\pm$ 1.72; commercial, 23.56 cm^2/plant $\pm$ 2.07; local, 20.66 cm^2/plant $\pm$ 1.86) (Figure 3.20).

E. elymoides per plant leaf area was significantly affected by PSSP, density, inoculum, and the PSSP x BRTE x density interaction (Table 3.4c). *E. elymoides* had significantly greater per plant leaf area in the local inoculum treatment (24.43 $\pm$ 2.69) than in the commercial (19.07 $\pm$ 2.27) and no-inoculum (17.10 $\pm$ 2.13) treatments. The significant PSSP x BRTE x density interaction shows that *E. elymoides* per plant leaf area was significantly affected by both the presence of *B. tectorum* and *P. spicatum*, and the density treatment (Figure 3.21). In the low density treatment, the presence of *B. tectorum* significantly reduced *E. elymoides* per plant leaf area regardless of whether *P. spicatum* was present, where as *P. spicatum* only significantly reduced *E. elymoides* per plant leaf

area in the three-species mixture. The presence of only *P. spicatum* reduced *E. elymoides* per plant leaf area (30.44 cm^2/plant $\pm$ 3.79), but not significantly (Figure 3.21). *E. elymoides* had greater per plant leaf area when both *P. spicatum* and *B. tectorum* were present than when only *B. tectorum* was present, but less than when only *P. spicatum* was present; these differences were not significant. In the high density treatment, *E. elymoides* had significantly reduced per plant leaf area when both other species were present compared to when only *P. spicatum* was present, but did not differ from when only *B. tectorum* was present or from the *E. elymoides* monoculture (Figure 3.21).

Phosphorus concentration and content

B. tectorum P concentration was significantly influenced by the PSSP x density, and PSSP x density x inoculum interactions (Table 3.4a). The significant PSSP x density x inoculum interaction shows the differential response of *B. tectorum* to *P. spicatum* presence across the inocula and density treatments. When *P. spicatum* was absent, *B. tectorum* P concentration did not differ significantly among all inocula and density treatment combinations. In contrast, when *P. spicatum* was present, the no-inoculum, high density mean was significantly greater than all other means except for the commercial inoculum, high density mean (Figure 3.22).

B. tectorum per plant P content was significantly affected by ELEL, density, inoculum, and the ELEL x inoculum, and density x inoculum interactions (Table 3.4a). The significant ELEL x inoculum interaction shows that the effect of the presence of *E. elymoides* depended on the inoculum treatment. For the commercial and local inoculum treatments, *B. tectorum* per plant P content was greater when *E. elymoides* was present versus when *E. elymoides* was absent. For the no-inoculum treatment, *B. tectorum* per

plant P content did not differ significantly from when *E. elymoides* was absent versus present (Figure 3.23). The significant density x inoculum interaction shows that the inoculum effect depended on density. Per plant P content for *B. tectorum* plants was significantly greater in the low density versus the high density treatment across all three inoculum treatments, but the difference was greatest for the local inoculum treatment (Figure 3.24).

P. spicatum P concentration and per plant P content were significantly affected by BRTE, inoculum, and the BRTE x inoculum interaction (Table 3.4b). *P. spicatum* P content was also significantly affected by the density main effect. *P. spicatum* had significantly greater per plant P content in the low density treatment (0.74 mg $\pm$ 0.09) than the high density treatment (0.35 mg $\pm$ 0.05).

Overall, *P. spicatum* had significantly greater P concentration in the local inoculum (1.84 mg/g $\pm$ 0.21) treatment than in the commercial (1.67 mg/g $\pm$ 0.20) and no-inoculum (1.51 mg/g $\pm$ 0.19) treatments, which did not differ significantly. In contrast, *P. spicatum* had significantly greater per plant P content in the commercial (0.64 mg $\pm$ 0.09) and local inoculum (0.59 mg $\pm$ 0.08) treatments than in the no-inoculum treatment (0.35 mg $\pm$ 0.05). The commercial and local inoculum treatments did not differ.

The significant BRTE x inoculum interactions for P concentration and per plant P content show that *P. spicatum* only responded to the absence of *B. tectorum* when inoculated; for the commercial and local inoculum treatments; *P. spicatum* had significantly greater P concentration and per plant P content when *B. tectorum* was absent versus present. In the no-inoculum treatment, *P. spicatum* had statistically equivalent P

concentrations and P contents when *B. tectorum* was absent versus present (Figures 3.25, 3.26).

E. elymoides P concentration and per plant P content were significantly affected by BRTE and inoculum (Table 3.4c). *E. elymoides* per plant P content was also significantly affected by the density main effect. *E. elymoides* had significantly greater P concentration and per plant P content when *B. tectorum* was absent versus present. Its P concentration and per plant P content were significantly greater in the commercial and local inoculum treatments than in the no-inoculum treatment (Table 3.5). *E. elymoides* had significantly greater P content in the low density (0.69mg $\pm$ 0.06) than in the high density (0.33mg $\pm$ 0.03) treatment.

B. tectorum and *P. spicatum* P concentrations were not correlated with SDM (r^2= -0.01431 P =0.8905; r^2=0.21495 P =0.0365; respectively), while *E. elymoides* P concentrations were positively correlated with SDM (r^2=0.27307 P =0.0075).

Discussion

Root responses to mycorrhizae

Root morphology measurements give different predictions regarding competitive ability. Specific root lengths of monocultures suggest that *B. tectorum* should have been the best competitor and *E. elymoides* should have been a better competitor than *P. spicatum* for soil resources because their roots had a greater surface to volume absorptive area for nutrients and water. In contrast, the per plant root length of monocultures suggests that the perennials should have been comparable competitors (comparable absorptive surface area) and that *B. tectorum* should be a better competitor than both perennials for soil resources in the local and commercial inoculum treatments. However,

in the no-inoculum treatment *B. tectorum* per plant root length was not greater than *E. elymoides*, thus based on absorptive surface area alone, *B. tectorum* and *E. elymoides* should have been comparable competitors in the no-inoculum treatment.

Both *B. tectorum* per plant root length and whole pot RDM were significantly greater in the local inoculum treatment where as they were equivalent in the no-inoculum and commercial inoculum treatments resulting in similar SRL between the three inoculum treatments.

The difference in root:shoot ratios of commercial and local inoculum treatments may be explained by the level of root colonization. All three species were colonized more by commercial AMF than local AMF. The commercially inoculated plants (compared to the locally inoculated plants) would have had a greater carbon demand and more carbon would have been allocated to the roots. The local inoculum had a lower colonization level (less carbon demand); therefore, carbon gained could be allocated to shoots rather than roots (Allen, 1996). The significantly lower root:shoot ratios in the local inoculum treatment compared to the no-inoculum treatment may indicate that local AMF were able to increase growth via greater P uptake. Greater growth in the local inoculum treatment is evident for *P. spicatum*. The AMF may have increased growth via increased P nutrition in the perennials (Koide, 1993), but *B. tectorum*'s benefits from AMF were not due to greater P status. *B. tectorum* may have benefited from AMF via increased plasticity or by greater N status, which was not measured in this study.

Despite having lower infectivity and colonization than the commercial inoculum, the local inoculum often had the greatest effect on plant morphology and physiology. This shows that infectivity and root colonization do not necessarily correspond to

effectiveness; possibly the local AMF had greater soil colonization (Auge *et al.*, 2007). A subsequent study may look at whether the infectivity and effectiveness of the two inocula changed in the field.

The significant inoculum x density interaction shows that inoculation, especially with local inoculum, was beneficial for whole pot RDM. However, differences between local and commercial inoculum were only evident in the high density treatment. This suggests that while the commercial inoculum does benefit the species in both density treatments, local inoculum is more beneficial when competition is greater.

B. tectorum whole pot RDM (in monoculture) benefited the most from local inoculum. *P. spicatum* responded to both the commercial and local inoculum treatment, whereas *E. elymoides* monoculture whole pot RDM did not respond to the inoculum treatment. It is unlikely that the lack of a mycorrhizal effect is due to pathogens: (1) both the commercial and local inoculum lacked an effect (2), the *E. elymoides* roots appeared healthy, (3) the commercial and local inoculum both had an effect on *P. spicatum*, and (4) the inoculum had a positive effect on other response variables. The commercial inoculum appears to benefit the perennials more than *B. tectorum* especially in the perennial mixture. These findings conflict with Benjamin & Allen (1987) who found that native (local) inoculum had a negative effect on *B. tectorum* RDM, and Rowe *et al.* (2007) who found that the *B. tectorum* was unresponsive to field (local) inoculum. These differences in results may be due to different environmental and biological conditions in the experiments such as different physical, chemical and biological soil properties, greenhouse conditions and intensity of competition (Ferrol *et al.*, 2004). However, similar to the *E. elymoides* whole pot RDM response to local inoculum, Rowe *et al.*

(2007) also found that *E. elymoides* was unresponsive to field (local) inoculum. In the present study, *E. elymoides* responsiveness to local inoculum depended on the response variable – in general the local inoculum was beneficial to *E. elymoides*. Rowe *et al.* (2007) calculated a mycorrhizal/control ratio using total dry weight, whereas in the present study the physiological measurements themselves were used to determine mycorrhizal effect; no ratio was used. Thus, whether an inoculum is determined to be effective may depend on how effectiveness is measured or calculated in a study.

Overall, the whole pot RDM trends found among the inoculum treatments for these species correspond well with the trends found in water use. The only incongruence was for locally inoculated *E. elymoides* monocultures, which used significantly greater water, but did not produce significantly greater whole pot RDM. SDM per pot corresponded even better than whole pot RDM to the water use for the inoculum trends found within each species, suggesting that whole pot SDM production may have driven water use more than whole pot RDM production.

Root WUE for all three species' monocultures was reduced in the local inoculum treatment, which might indicate that soil colonization was greater in the local inoculum treatment even though root colonization was lower in the local than in the commercial inoculum treatment (Augé *et al.* 2007). Plants were less efficient at turning water into root biomass when inoculated by local AMF because the carbon in the roots was going towards hyphae development. With more extensive hyphae in the soil, the local inoculated plants were able to increase photosynthesis and carbon allocation to the shoots leading to the lowest root:shoot ratios.

Aboveground responses to mycorrhizae and competition

P. spicatum had the greatest mycorrhizal colonization, but the colonization tended to be beneficial only when *B. tectorum* was absent. *E. elymoides* also consistently benefited from inoculation, but it tended to benefit regardless of the invasive's presence. Although *B. tectorum* had extremely low colonization by the local AMF, this inoculation greatly benefited the invasive. The low local AMF colonization may indicate that other microflora/fauna in the inoculum are beneficial to *B. tectorum,* or as seen in other studies, colonization level does not correspond to effectiveness of the local AMF (Ahiabor & Hirata, 1994; Mohammad *et al.*, 2004; Smith *et al.*, 2004; Li *et al.*, 2005). Possibly the local AMF had greater soil colonization (Augé *et al.*, 2007) than the commercial inoculum resulting in greater effectiveness. However, for the perennials, the local inoculum was not always more effective than the commercial inoculum. In some cases, the commercial inoculum appeared more beneficial.

P. spicatum per plant SDM did not change in response to *B. tectorum* within either the no-inoculum or commercial inoculum treatment. In contrast, in the local inoculum treatment, *P. spicatum* had reduced per plant SDM when grown with *B. tectorum*. Since in the absence of *B. tectorum* competition, local inoculum increased *P. spicatum* per plant SDM relative to the no-inoculum treatment, but in the presence of *B. tectorum, P. spicatum* per plant SDM was similar to the no-inoculum treatment; the local inoculum may only be more beneficial in the absence of *B. tectorum*. The reason there was reduced per plant SDM within the local inoculum treatment in the presence of *B. tectorum* may be that the local inoculum benefits *B. tectorum* to a greater degree than it does *P. spicatum* resulting in greater competition and thus reduced *P. spicatum* per plant

SDM. *P. spicatum* per plant SDM did not change in response to *B. tectorum* within the commercial inoculum treatment possibly due to the fact that the commercial inoculum was not as beneficial to *B. tectorum,* so *P. spicatum* maintained its per plant SDM because *B. tectorum* competition was not as great as in the local inoculum treatment or because the commercial inoculum increased the competitiveness of *P. spicatum.* Thus, the commercial inoculum may be more beneficial to *P. spicatum* under competition due to a greater direct positive AMF effect on the native or it may be more beneficial due to an indirect positive AMF effect. Allen & Allen (1984) found that soil inoculum (but not spore inoculum) ameloriated the competitive effect of *Salsola kali* on *Bouteloua gracilis* SDM. Similarly, Benjamin & Allen (1987) found that inoculum ameloriated the effect of competitors such as *B. tectorum* on *Agropyron dasystachyum,* but the degree of ameloriation depended on the identity of the competitor.

For *P. spicatum,* a positive direct effect (ameloriation) would likely be that commercial AMF increase carbon fixation and nutrient uptake more than local inoculum in the presence of the invasive. An indirect effect would be that the commercial inoculum is not as beneficial to *B. tectorum* as the local inoculum, making *B. tectorum* less competitive in the commercial inoculum treatment resulting in increased growth by *P. spicatum*. The direct or indirect effect of commercial mycorrhizae could be explained by host specificity. Although both *P. spicatum* and *B. tectorum* may easily be colonized by the commercial AMF, particular AMF species in the commercial mycorrhizae blend may be more compatible with the native than the invasive (Hart *et al.*, 2003; Scheublin *et al.*, 2007). The change in *P. spicatum* per plant SDM may be due to a change in the dominant AMF in the soil when *B. tectorum* and *P. spicatum* are in mixture than when

not in mixture rather than due to direct interspecific competition (Allen & Allen 1990; Bever, 1999; Eom *et al.,* 2000). The host plant may change the dominant AMF by affecting the AMF's sporulation, growth, and survival (Eom *et al.*, 2000). Host specificity combined with colonization level and the associated balance between carbon drain and mycorrhizal benefits may explain the different effects of the two inocula on the two plant species (Allen & Allen 1990; Hart *et al.,* 2003).

E. elymoides response to *P. spicatum* also depended on the inoculum treatment. *E. elymoides* per plant SDM was only significantly reduced by *P. spicatum* presence in the no-inoculum treatment. However, there was a nonsignificant trend for *E. elymoides*: per plant SDM tended to be greater in the presence of *P. spicatum* in the commercial treatment but greater in the absence of *P. spicatum*'s in the local inoculum treatment. This might suggest that the commercial inoculum is more beneficial than the local inoculum to *E. elymoides* per plant SDM when competing with *P. spicatum* (Hart *et al.* 2003; Scheublin *et al.* 2007). The *E. elymoides* per plant SDM response did not appear to be an effect of *P. spicatum* shading out *E. elymoides* since *P. spicatum* had 52% more per plant SDM than *E. elymoides* in the commercial inoculum treatment and only 7% more per plant SDM than *E. elymoides* in the local inoculum treatment.

In the low density treatment, *E. elymoides* per plant SDM seemed to be more affected by the presence of *B. tectorum* and *P. spicatum,* whereas in the high density treatment it seemed to respond more to resource availability regardless of whether competition was intraspecific or interspecific. The greater root:shoot ratios and root WUE in the high density treatment suggests greater soil resource competition in the high density treatment. *E. elymoides* per plant leaf area also showed the same pattern as per

plant SDM to the presence of the two other species in the low and high density treatments. Greater competition for soil resources and light might reduce *E. elymoides* per plant leaf area leading to less SDM production.

Both *E. elymoides* and *B. tectorum* SLA depended on competitor presence. *B. tectorum* reduced *E. elymoides* SLA in the high density treatment compared to the low density treatment; while the presence of *P. spicatum* increased *B. tectorum* SLA, but only in the no-inoculum treatment. *P. spicatum* SLA did not depend on competitor presence, but only on inoculum. *P. spicatum* had increased SLA in the local inoculum treatment.

B. tectorum per plant SDM, leaf area, and P content increased in the presence of *E. elymoides* only when inoculated. *E. elymoides* may be stimulating *B. tectorum's* use of mycorrhizae (Schwab & Loomis, 1987). However, unlike Schwab & Loomis' study, the intensity of competition did not alter *B. tectorum*'s response to mycorrhizae, but rather the identity of the competitor did (Pendleton & Smith, 1983).

Overall, *B. tectorum* had the greatest per plant tiller production in the local inoculum treatment especially when both perennials were present. When only *P. spicatum* was present, *B. tectorum* did not respond to either inoculum treatment, whereas when only *E. elymoides* was present *B. tectorum* benefited from commercial inoculum (the local inoculum treatment was similar to both the no and commercial inoculum treatments). Possibly *B. tectorum* did not respond to either inoculum when grown only with *P. spicatum* because *P. spicatum* per plant tiller production also did not respond to either inoculum, so a competitive response by *B. tectorum* was not triggered. In contrast, when grown only with *E. elymoides, B. tectorum* had the greatest per plant tiller production in the commercial inoculum treatment. However, *E. elymoides* did not

respond to the commercial inoculum, only to the local inoculum treatment (increased per plant tiller production compared to the no-inoculum treatment). The commercial inoculum effect on *B. tectorum* may be independent of its effect on *E. elymoides*. Possibly when grown with both perennials, *B. tectorum* has the greatest per plant tiller production in the local inoculum treatment due to a combined effect of *E. elymoides* having greatest per plant tiller production in the local inoculum treatment and there being proportionally more heterospecifics (six perennial plants versus two *B. tectorum* plants). Thus, greater interspecific competition might result in greater use of mycorrhiza by *B. tectorum* (Schwab & Loomis, 1987). However, in this study greater interspecific competition was due to the greater competitive ability of *E. elymoides* and greater proportion of heterospecifics where as in Schwab & Loomis' study, greater interspecific competition was due to only a greater proportion of heterospecifics. The greater mycorrhizal benefit seen in *B. tectorum* during interspecific competition is in contrast to the general idea that inoculation shifts the competitive balance towards the more mycorrhizal dependent species (Allen & Allen, 1990; Hartnett *et al.*, 1993; Hart *et al.*, 2003), either by providing greater benefit to the more dependent species (Scheublin *et al.*, 2007) or by negatively affecting the non-mycorrhizal species (Ruotsalainen & Aikio, 2004).

Possibly the results found by Schwab & Loomis (1987) and this study can be explained by translocation of nutrients or photosynthate through mycelial networks between the invasive and the native(s). Further research would need to be done because neither study looked at shared mycelial networks (Marler *et al.*, 1999; Hart *et al.*, 2003). Marler *et al.* (1999) found that the invasive *Centaurea maculosa* benefited from AMF

when grown with *Festuca idahoensis*, particularly when *F. idahoensis* was larger, but did not exhibit any benefits when grown only with conspecifics. They hypothesized this may be due to resources being transferred from the native to the invasive via hyphae. As the proportion of more mycorrhizae-dependent (*P. spicatum*) plants increased relative to *B. tectorum* plants in Schwab & Loomis' study, the opportunities for a shared mycelial network between the invasive and native likely increased. Although shared mycelial networks are documented, their importance in plant competition and coexistence is not well known (Allen & Allen 1990; Hartnett *et al.*, 1993; Smith & Read 1997; Hart *et al.*, 2003). A shift in the AMF species community structure when interspecific competition was greater might also explain the greater mycorrhizal benefit by *B. tectorum* (Allen & Allen 1990; Eom *et al.*, 2000).

All three species responded to greater water availability and soil resources and less light competition in the low density treatment by increasing per plant SDM and per plant leaf area. *B. tectorum* per plant tiller response to density varied depending on whether it was grown with either or both perennials. *B. tectorum* had greater per plant tiller production in the low density treatment versus the high density treatment. In the low density treatment when grown alone, *B. tectorum* is self-shading, which results in lower per plant tiller production, where as in mixture, *B. tectorum* neighbors were perennials, which had lower per plant leaf area and per plant SDM than the invasive, resulting in less shading by neighbors and greater tiller production by *B. tectorum*. In contrast, in the high density treatment, *B. tectorum* might be responding to lower water availability and soil resources (greater root mass in the soil), as well as greater shading (light competition).

High density and competition with *B. tectorum* reduced perennial per plant tiller numbers, probably due to both shading and greater competition for soil resources (water, nutrients, etc.). The lower root:shoot ratios of *B. tectorum* and greater shoot WUE suggest that it was more efficient at turning water and nutrients into shoot biomass, which would have resulted in a greater competitive effect by the invasive, especially in the high density treatment when less water and nutrients per plant were available.

Plant phosphorus status and mycorrhizae

All three species had significantly greater per plant P content in the local inoculum than the no-inoculum treatment, and the local inoculum treatment had either significantly greater per plant P content (*B. tectorum*) or statistically equivalent per plant P content (*P. spicatum* and *E. elymoides*) to the commercial inoculum treatment. The greater per plant P content may be due to greater P demand because per plant SDM was greater in the commercial and/or local inoculum treatments than in the no-inoculum treatment (Koide, 1993). All three species had equivalent P concentrations between the local and commercial inoculum treatments. The greater per plant P content, but statistically equivalent P concentration of *B. tectorum* in the local treatment compared to the commercial inoculum treatment can be explained by the greater per plant SDM in the local inoculum treatment than the commercial, which is evidence of the dilution effect (Jarrell & Beverly, 1981). The perennials had equivalent per plant P contents and per plant SDMs between the commercial and local inoculum treatments resulting in similar P concentrations between the two treatments.

The effect of *B. tectorum* on *P. spicatum* P concentration and per plant P content was a neutralizing of the AMF effect. Both commercial and local inocula increased P

concentration and per plant P content when *B. tectorum* was absent, relative to the no inoculum treatment, but when *B. tectorum* was present, the inoculum had no visible effect on P status. If AMF were not beneficial to *P. spicatum* when *B. tectorum* was present, it would be expected that *P. spicatum* per plant P content and concentration would decrease in the presence of *B. tectorum* compared to in the absence of *B. tectorum* in the no-inoculum treatment. *B. tectorum* had greater per plant: SDM, root length, and P content in the commercial and local inoculum treatments compared to the no-inoculum and at least, in monocultures, it had greater whole pot RDM in the local inoculum treatment. The greater growth and competitive ability of *B. tectorum* in the commercial and local inoculum treatments may be balancing out the positive effect of AMF on *P. spicatum* resulting in a net no change. This would indicate that the inocula are benefiting both *B. tectorum* and *P. spicatum*, but they are having a greater effect on *B. tectorum*.

P uptake was affected by density for all three species. However, *B. tectorum* response to the density treatment depended on its inoculation status. *B. tectorum* seems to have greater access to water and/or nutrients in the local inoculum treatment. At least in monoculture, *B. tectorum* had greater water use in the local inoculum treatment, which corresponded to greater per plant and whole pot SDM and whole pot RDM, which would lead to a greater demand for P. Thus, if conditions were beneficial for the mutualistic association – which they appear to be – the greater P demand would lead to greater P uptake in the local inoculum treatment (Koide, 1993).

B. tectorum competitively reduced *E. elymoides* P shoot content and concentration. *E. elymoides* P concentration was positively correlated with per plant

SDM indicating that *B. tectorum* may have competitively reduced *E. elymoides* per plant SDM by negatively impacting its P uptake or vice versa.

Overall, it appears that inoculation benefited all three species. With interspecific competition, the local inoculum had a greater positive effect on *B. tectorum* than on the perennials resulting in an indirect, negative AMF effect on the perennials. In some cases, it appears that the commercial inoculum benefited the perennials more than the local inoculum during interspecific competition. These findings contrast with a study done by Rowe *et al.* (2007) that found that both *B. tectorum* and *E. elymoides* had a negative response to inoculum from a local site. Other studies have also found a negative or neutral effect of mycorrhizae on *B. tectorum* (Allen 1984, 1988; Benjamin & Allen, 1987). However, the mycorrhizal effect on *B. tectorum* in these studies may be due to low or no interspecific competition. The effect of mycorrhizae may only become beneficial for the invasive under intense interspecific competition (Schwab & Loomis, 1987) and may also depend on its competitors' identities. This highlights the fact that the biological conditions on a site are important to consider when choosing inocula for restoration projects (van der Heijden *et al.*, 1998). Furthermore, low P and environmental stress may have caused the invasive to positively respond to mycorrhizae. Thus, like the perennial grasses, *B. tectorum* response to mycorrhizae depends both on abiotic and biotic conditions. The artificial conditions created in the greenhouse may have caused *B. tectorum* to benefit more from mycorrhizae than it would in the field. In a natural system, the perennials, compared to *B. tectorum*, may show a greater positive response to the local inoculum than the commercial inoculum. A similar study needs to

be performed in the field to see if the dynamics between mycorrhizae, the invasive and the natives changes.

In conclusion, inoculation with either inocula benefited all three species, but in general the local inoculum had a greater effect than the commercial inoculum. During interspecific competition, the local inoculum benefited *B. tectorum* more than the perennials. In some cases the commercial inoculum was most beneficial to the perennials during interspecific competition; for example *P. spicatum* per plant SDM response to *B. tectorum* and *E. elymoides* SDM response to *P. spicatum*.

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Table 3.1 P-values for fixed effects (species, density, and inoculum) of percent root colonization, whole pot root dry mass (RDM) , specific root length (SRL), root length (RL) per plant, total water use efficiency (WUE), shoot WUE, root WUE, total water use (per pot), root:shoot ratio, and whole pot shoot dry mass (SDM). Significant p-values at the 0.01 level are indicated in bold.

Fixed Effect	Percent Root Colonization	Whole Pot RDM	SRL	RL per plant	Total WUE	Shoot WUE	Root WUE	Total Water Use	Root:shoot ratio	Whole Pot SDM
Species	**0.0038**	**0.0007**	**<0.0001**	**<0.0001**	**0.0026**	**0.0013**	0.1831	**<0.0001**	**<0.0001**	**<0.0001**
Density	0.2110	**<0.0001**	0.3981	**<0.0001**	0.4316	0.0226	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Inoculum	**<0.0001**	**<0.0001**	**0.0001**	**0.0003**	0.2579	0.6710	**0.0019**	**<0.0001**	**0.0008**	**<0.0001**
Species x Density	0.0957	0.1684	0.7578	**0.0059**	0.0262	0.4568	0.0361	0.2302	0.1767	0.3338
Species x Inoculum	**0.0094**	**<0.0001**	**<0.0001**	**<0.0001**	0.9482	0.6009	0.0504	**0.0025**	0.1211	**<0.0001**
Density x Inoculum	0.2353	**0.0034**	0.0231	0.8953	0.6812	0.2758	0.3804	0.1372	0.4005	**0.0046**
Species x Density x Inoculum	0.0182	0.2131	0.0283	0.0409	0.6505	0.9695	0.2701	0.5602	0.7068	0.4702

Table 3.2 Total WUE and shoot WUE for species (*B. tectorum*, BRTE; *P. spicatum*, PSSP; and *E. elymoides*, ELEL) monocultures and mixtures. Significant differences within a given treatment (each column) are indicated by different letters.

Monoculture or Mixture	Total Water Use Efficiency	Shoot Water Use Efficiency
BRTE	3.51E-03 ± 1.80E-04[a]	2.57E-03 ± 2.20E-04[a]
PSSP	2.90E-03 ± 1.84 E-04 [bc]	1.81E-03 ± 1.57E-04[b]
ELEL	2.81E-03 ± 1.91E-04 [bc]	1.99E-03 ± 1.73E-04[b]
BRTE and PSSP	2.97E-03 ± 1.80E-04 [abc]	1.92E-03 ± 1.64E-04[b]
BRTE and ELEL	3.10E-03 ± 1.80E-04 [abc]	2.10E-03 ± 1.79E-04[ab]
PSSP and ELEL	2.64E-03 ± 1.84E-04[c]	1.76E-03 ± 1.50E-04[b]
BRTE, PSSP, and ELEL	3.28E-03 ± 1.80E-04[ab]	2.18E-03 ± 1.86E-04[ab]

Table 3.3 Least squares means of root:shoot ratios for inoculum, density, and species (*B. tectorum*, BRTE; *P. spicatum*, PSSP; and *E. elymoides*, ELEL) treatments. Significant differences within a given treatment are indicated by different letters.

Treatment	LSMean	Standard Error
Inoculum		
No Inoculum	0.47^{b}	0.05
Commercial Inoculum	0.51^{a}	0.05
Local Inoculum	0.41^{c}	0.05
Density		
Low Density	0.38^{b}	0.04
High Density	0.56^{a}	0.05
Species		
BRTE	0.31^{d}	0.05
PSSP	0.57^{a}	0.06
ELEL	0.47^{abc}	0.06
BRTE and PSSP	0.50^{abc}	0.06
BRTE and ELEL	0.44^{c}	0.05
PSSP and ELEL	0.52^{ab}	0.06
BRTE, PSSP, and ELEL	0.45^{bc}	0.05

Table 3.4a P-values for fixed effects (*P. spicatum*, PSSP; *E. elymoides*, ELEL; Density; and Inoculum and all associated interactions) for *B. tectorum* (BRTE) per plant shoot dry mass (SDM), per plant tiller number, specific leaf area (SLA), per plant leaf area (LA), P concentration (P Conc.), and per plant P content. Significant parameters at P<0.01 are indicated in bold.

Fixed Effect	BRTE Per Plant SDM	BRTE Per Plant Tiller #	BRTE SLA	BRTE Per Plant LA	BRTE P Conc	BRTE Per Plant P Content
PSSP	0.1787	0.0888	0.0499	0.0405	0.1183	0.0609
ELEL	**0.0007**	**0.0006**	0.7625	**<0.0001**	0.8001	**0.0014**
Density	**<0.0001**	**<0.0001**	0.5196	**<0.0001**	0.1014	**<0.0001**
Inoculum	**<0.0001**	**<0.0001**	**0.0034**	**<0.0001**	0.5627	**<0.0001**
PSSP x ELEL	0.5744	0.7389	0.3867	0.2920	0.3041	0.2709
PSSP x Density	0.5167	0.6062	0.9713	0.8864	**0.0059**	0.2520
PSSP x Inoculum	0.1841	0.4031	**0.0091**	0.2219	0.1798	0.3684
ELEL x Density	0.5283	0.0564	0.2437	0.1992	0.5838	0.2098
ELEL x Inoculum	**<0.0001**	**0.0039**	0.2101	**<0.0001**	0.6219	**0.0013**
Density x Inoculum	0.1741	0.3893	0.4945	**0.0023**	0.0238	**0.0040**
PSSP x ELEL x Density	0.0125	**0.0002**	0.4544	0.0187	0.9978	0.0814
PSSP x ELEL x Inoculum	0.1084	**0.0024**	0.5388	0.1175	0.0286	0.0149
PSSP x Density x Inoculum	0.4931	0.7366	0.0698	0.2691	**0.0019**	0.1191
ELEL x Density x Inoculum	0.2212	0.6545	0.9274	0.3047	0.2896	0.6900
PSSP x ELEL x Density x Inoculum	0.3034	0.0998	0.0998	0.7801	0.0226	0.3491

Table 3.4b P-values for fixed effects (*E. elymoides*, ELEL; *B. tectorum*, BRTE; Density; and Inoculum and all associated interactions) for *P. spicatum* (PSSP) per plant shoot dry mass (SDM), per plant tiller number, specific leaf area (SLA), per plant leaf area (LA), P concentration (P Conc.), and per plant P content. Significant parameters at P<0.01 are indicated in bold.

Fixed Effect	PSSP Per Plant SDM	PSSP Per Plant Tiller #	PSSP SLA	PSSP Per Plant LA	PSSP P Conc.	PSSP Per Plant P Content
ELEL	0.9803	0.4005	0.3256	0.9055	0.5811	0.4718
BRTE	**<0.0001**	**0.0032**	0.8454	**<0.0001**	**0.0003**	**<0.0001**
Density	**<0.0001**	**<0.0001**	0.4670	**<0.0001**	0.1311	**<0.0001**
Inoculum	**<0.0001**	0.0540	**<0.0001**	**<0.0001**	**0.0058**	**<0.0001**
ELEL x BRTE	0.1709	0.2320	0.6306	0.0367	0.0311	0.0126
ELEL x Density	0.5552	0.3293	0.7213	0.6283	0.9908	0.3856
ELEL x Inoculum	0.1139	0.6288	0.1394	0.7223	0.7977	0.7167
BRTE x Density	0.1692	0.2131	0.7900	0.1759	0.3273	0.1428
BRTE x Inoculum	**0.0005**	0.2087	0.3497	**0.0039**	**<0.0001**	**<0.0001**
Density x Inoculum	0.0528	0.1318	0.2413	0.2391	0.8240	0.3699
ELEL x BRTE x Density	0.4232	0.9510	0.7031	0.5180	0.9366	0.8776
ELEL x BRTE x Inoculum	0.4291	0.0796	0.9498	0.1375	0.1204	0.3794
ELEL x Density x Inoculum	0.4745	0.6973	0.6547	0.9741	0.1230	0.9979
BRTE x Density x Inoculum	0.3517	0.0321	0.0616	0.6406	0.2204	0.3770
ELEL x BRTE x Density x Inoculum	0.5728	0.2371	0.6048	0.8902	0.6101	0.4291

Table 3.4c P-values for fixed effects (*P. spicatum*, PSSP; *B. tectorum*, BRTE; Density; and Inoculum and all associated interactions) for *E. elymoides* (ELEL) per plant shoot dry mass (SDM), per plant tiller number, specific leaf area (SLA), per plant leaf area (LA), P concentration (P Conc.), and per plant P content. Significant parameters at P<0.01 are indicated in bold.

Fixed Effect	ELEL Per Plant SDM	ELEL Per Plant Tiller #	ELEL SLA	ELEL Per Plant LA	ELEL P Conc	ELEL Per Plant P Content
PSSP	0.1935	0.2788	0.0579	0.6734	0.8913	0.8073
BRTE	**<0.0001**	**0.0005**	0.6534	**<0.0001**	**<0.0001**	**<0.0001**
Density	**<0.0001**	**<0.0001**	0.0407	**<0.0001**	0.1608	**<0.0001**
Inoculum	**0.0022**	**0.0008**	0.0578	**0.0004**	**0.0033**	**0.0001**
PSSP x BRTE	0.0246	0.4332	0.0657	0.1595	0.8583	0.1935
PSSP x Density	0.7295	0.3923	0.6168	0.9733	0.6448	0.8637
PSSP x Inoculum	**0.0072**	0.0166	0.1466	0.0272	0.8077	0.0835
BRTE x Density	**0.0051**	0.3727	**0.0017**	0.1582	0.8643	0.1038
BRTE x Inoculum	0.6589	0.1202	0.0484	0.2993	0.0139	0.0523
Density x Inoculum	0.3398	0.6706	0.8135	0.8726	0.3197	0.2842
PSSP x BRTE x Density	**0.0062**	0.1810	0.8789	**0.0079**	0.2297	0.5834
PSSP x BRTE x Inoculum	0.7017	0.0924	0.1358	0.5052	0.3500	0.4247
PSSP x Density x Inoculum	0.6490	0.1881	0.9111	0.5500	0.5796	0.6137
BRTE x Density x Inoculum	0.2545	0.8793	0.3623	0.2237	0.5294	0.5887
PSSP x BRTE x Density x Inoculum	0.9163	0.0211	0.4481	0.7602	0.6734	0.8884

Table 3.5 *E. elymoides'* shoot P content (per plant) and concentration least squares means ± 1 SE as affected by inocula and presence of *B. tectorum* (BRTE). Significant differences within a given treatment are indicated by different letters.

Treatment	P content per plant (mg/plant)	P concentration (mg/g)
Inoculum		
No Inoculum	0.36 ± 0.04^{b}	1.67 ± 0.15^{b}
Commercial Inoculum	0.51 ± 0.05^{b}	2.03 ± 0.16^{b}
Local Inoculum	0.61 ± 0.06^{a}	2.06 ± 0.15^{a}
BRTE		
Absent	0.65 ± 0.05^{a}	2.17 ± 0.14^{a}
Present	0.36 ± 0.04^{b}	1.68 ± 0.15^{b}

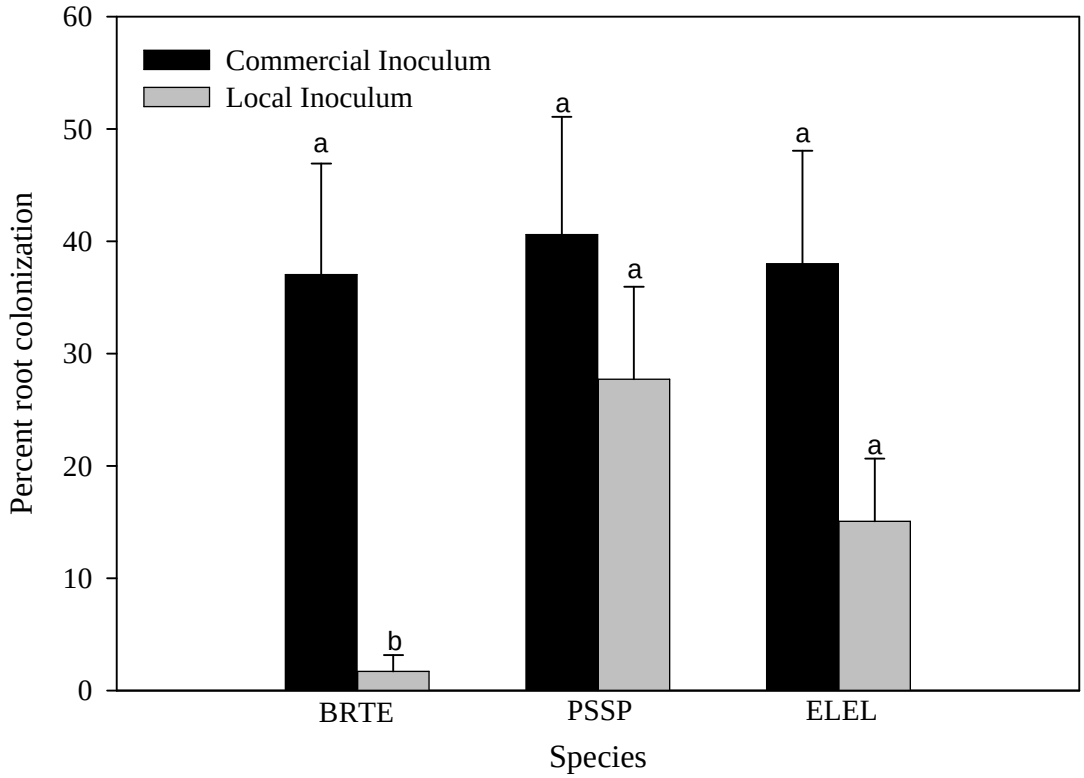

Figure 3.1 Local and commercial arbuscular mycorrhizal fungi percent root colonization for *B. tectorum*, *P. spicatum*, and *E. elymoides*. Bars represent means for four replicates with error bars representing + 1 standard error. Percent root colonization values, a measure of the percent of the root system colonized by mycorrhizae, are for colonization 44 days after the water stress phase of the experiment began. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

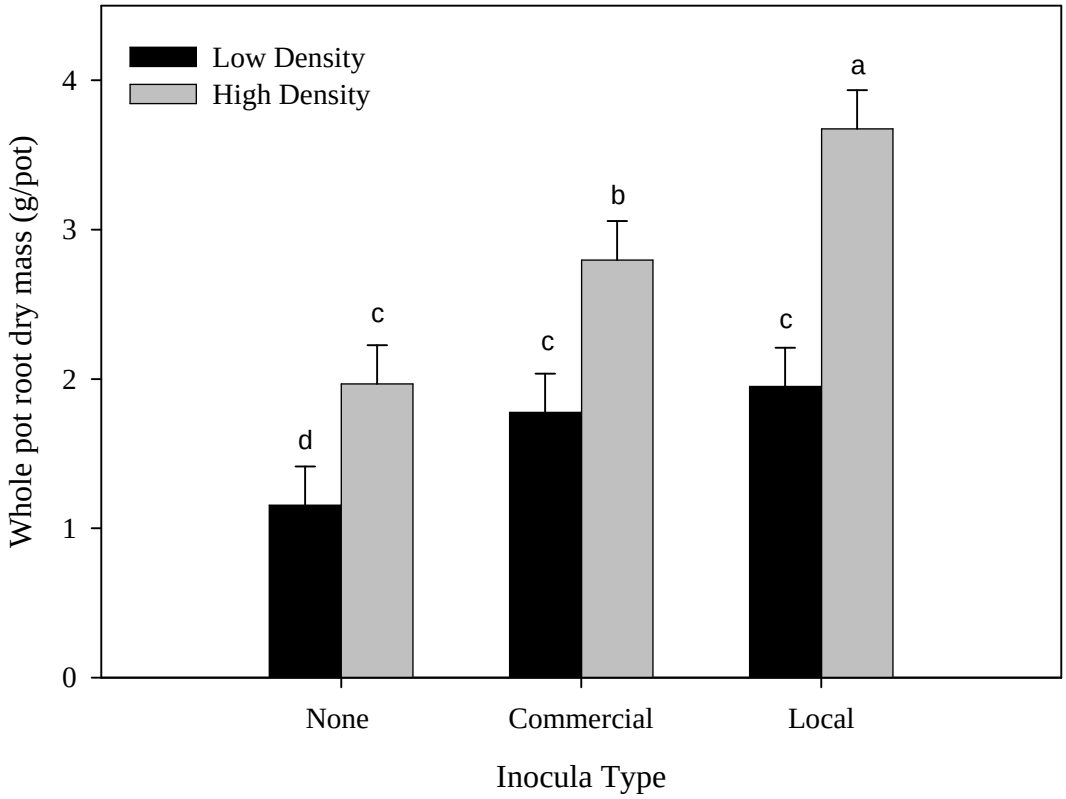

Figure 3.2 The effect of inocula type and intraspecific competition on whole pot root dry mass. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

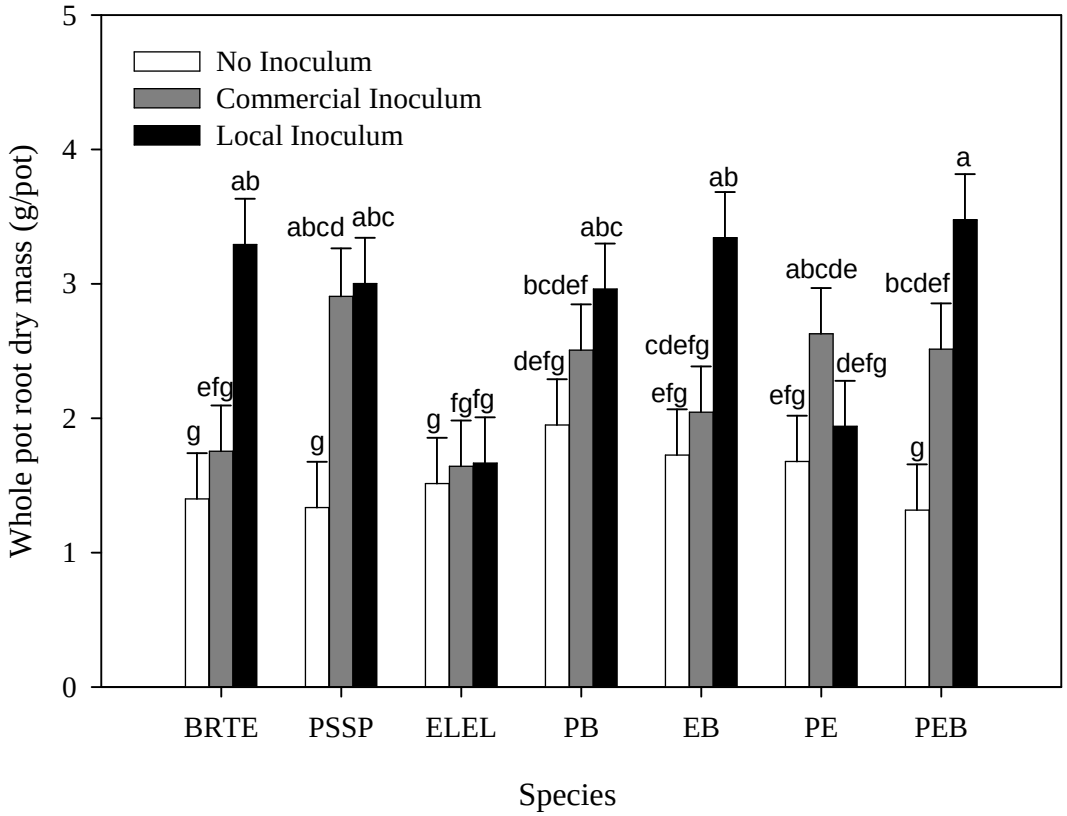

Figure 3.3 The effect of inocula type on whole pot root dry mass of 3 species monocultures and 4 species mixtures. Bars represent means for four replicates with error bars representing + 1 standard error. Species monocultures are *B. tectorum* (BRTE), *P. spicatum* (PSSP) and *E. elymoides* (ELEL). Species mixtures are *P. spicatum* and *B. tectorum* (PB), *E. elymoides* and *B. tectorum* (EB), *P. spicatum* and *E. elymoides* (PE) and three species mixture (PEB). Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

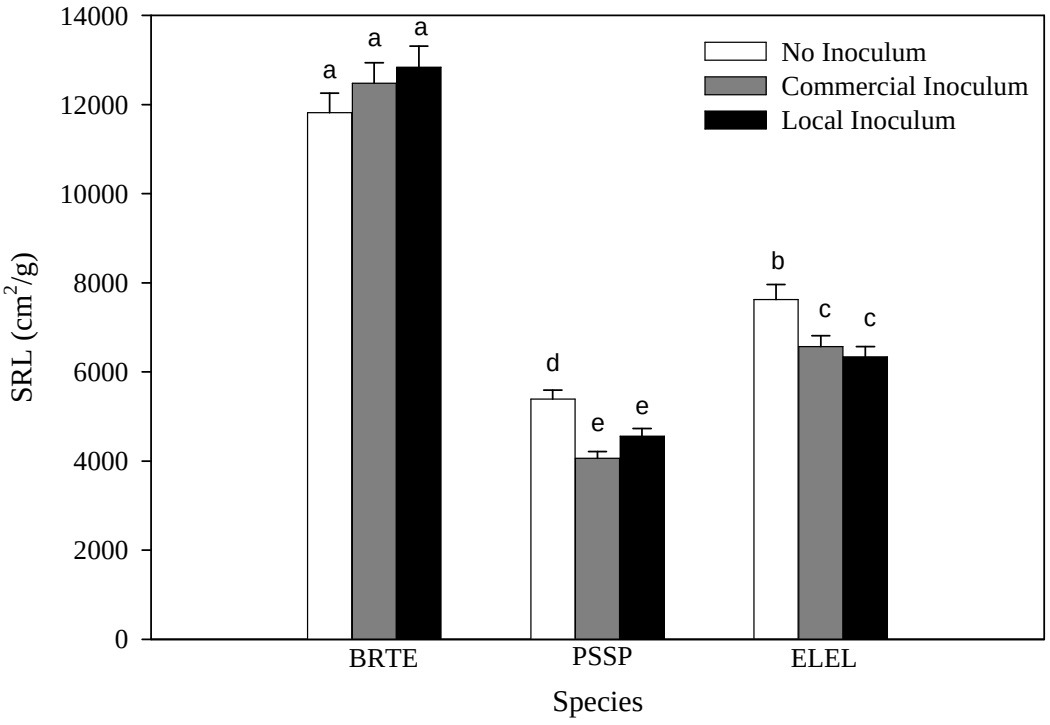

Figure 3.4 The effect of inocula type on specific root length of *B. tectorum*, *P. spicatum*, and *E. elymoides* monocultures. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

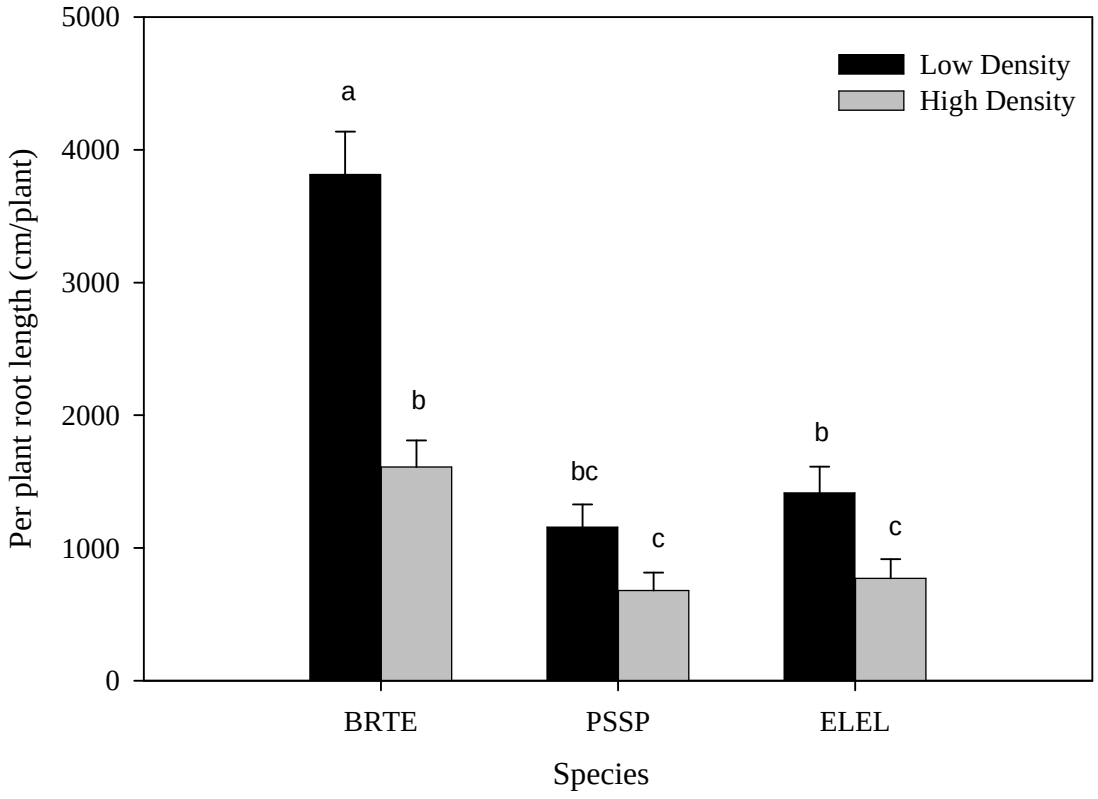

Figure 3.5 The effect of intraspecific competition on per plant root length of *B. tectorum* (BRTE), *P. spicatum* (PSSP), and *E. elymoides* (ELEL) monocultures. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

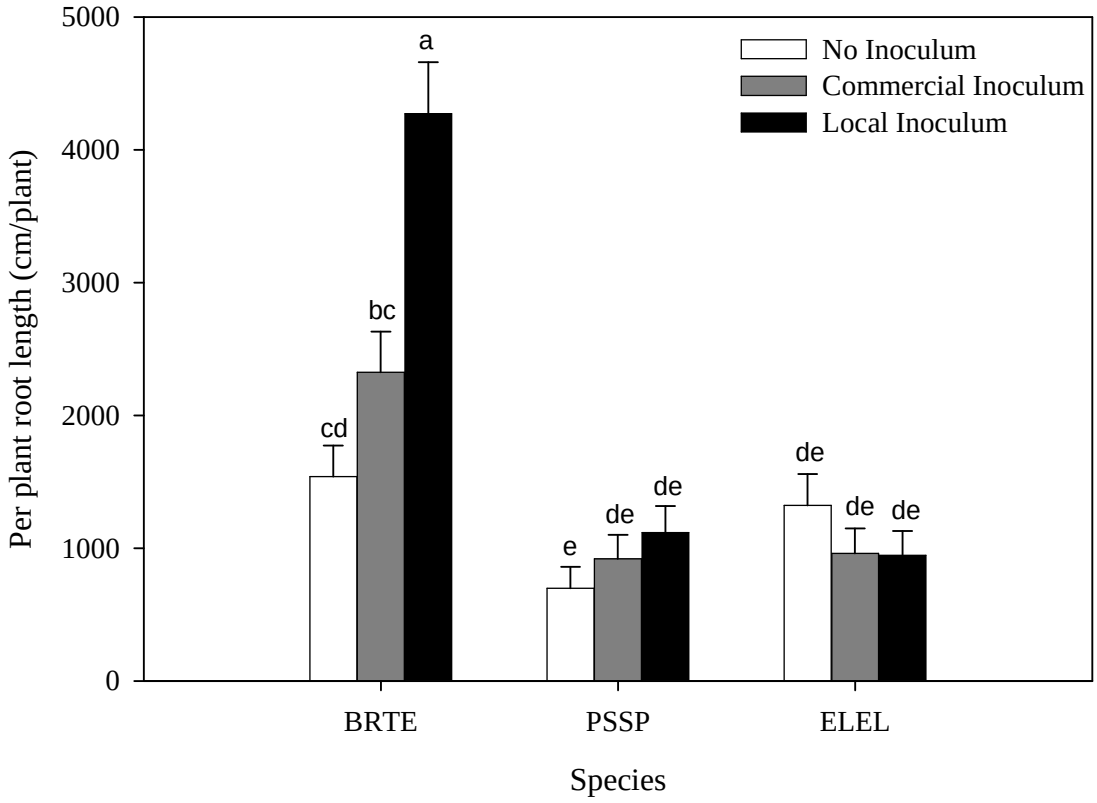

Figure 3.6 The effect of inocula type on per plant root length of *B. tectorum* (BRTE), *P. spicatum* (PSSP), and *E. elymoides* (ELEL) monocultures. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

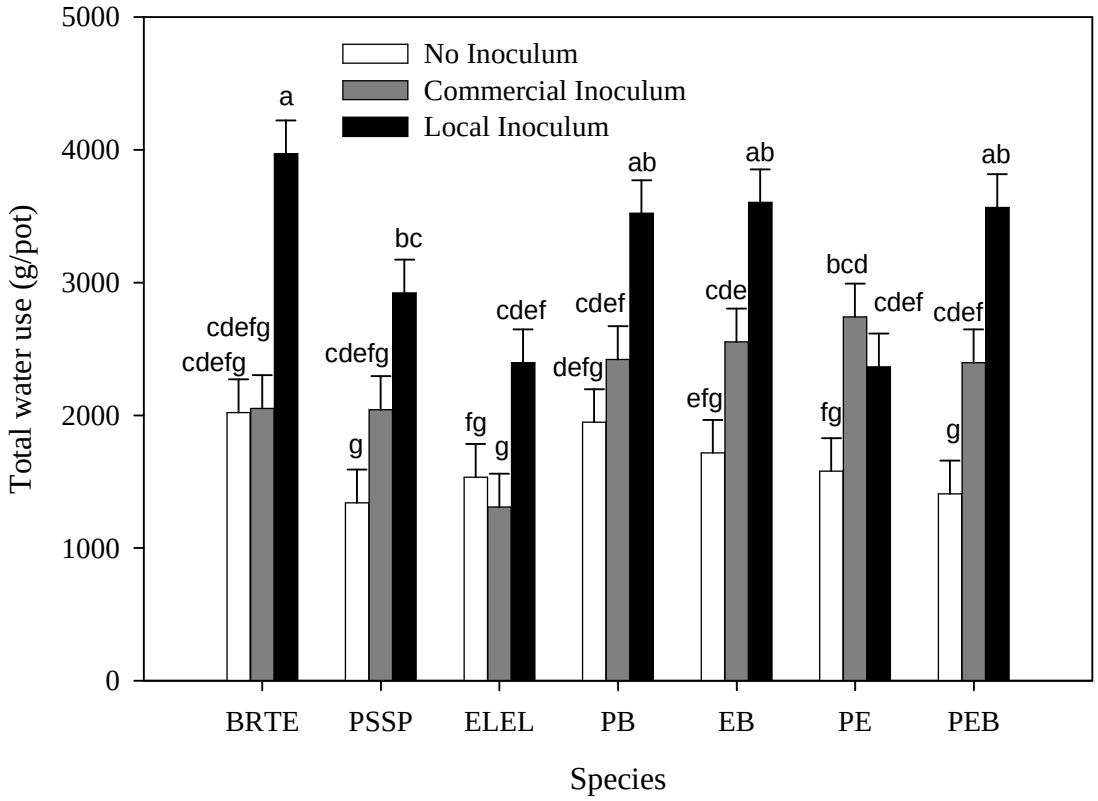

Figure 3.7 The effect of inocula type on total water use of 3 species monocultures and 4 species mixtures. Bars represent means for four replicates with error bars representing + 1 standard error. Species monocultures are *B. tectorum* (BRTE), *P. spicatum* (PSSP) and *E. elymoides* (ELEL). Species mixtures are *P. spicatum* and *B. tectorum* (PB), *E. elymoides* and *B. tectorum* (EB), *P. spicatum* and *E. elymoides* (PE) and three species mixture (PEB). Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

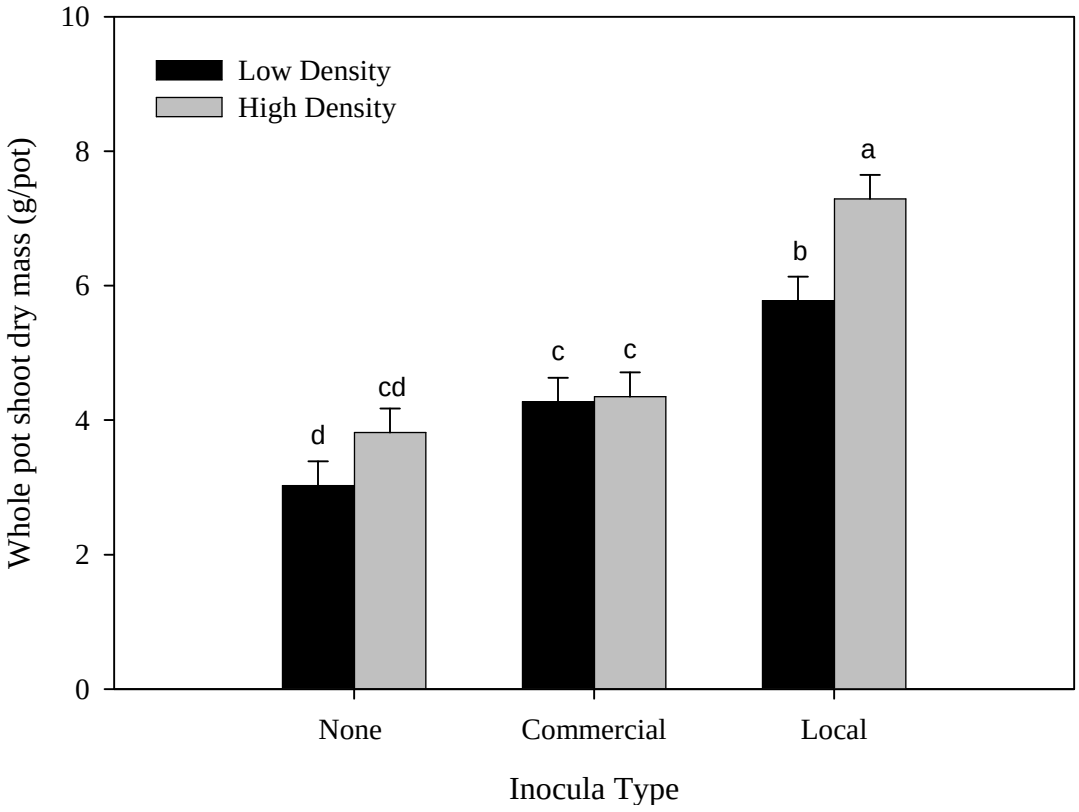

Figure 3.8 The effect of inocula type and intraspecific competition on whole pot shoot dry mass of *B. tectorum*, *P. spicatum*, and *E. elymoides* monocultures. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

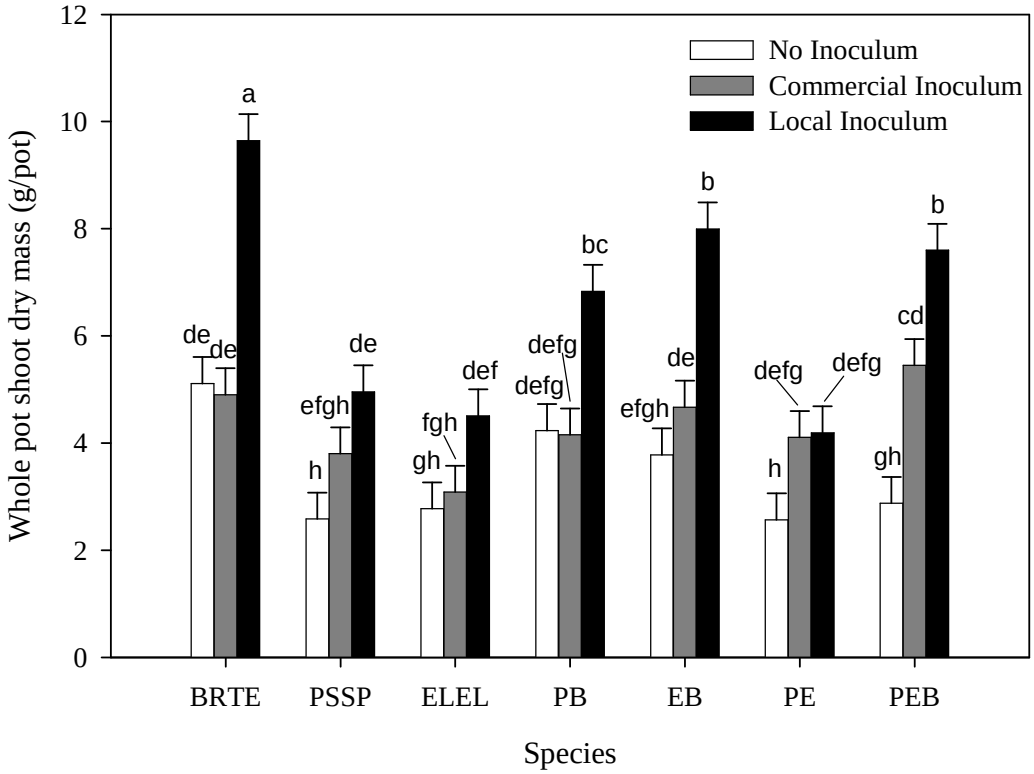

Figure 3.9 The effect of inocula type on whole pot shoot dry mass of 3 species monocultures and 4 species mixtures. Bars represent means for four replicates with error bars representing + 1 standard error. Species monocultures are *B. tectorum* (BRTE), *P. spicatum* (PSSP) and *E. elymoides* (ELEL). Species mixtures are *P. spicatum* and *B. tectorum* (PB), *E. elymoides* and *B. tectorum* (EB), *P. spicatum* and *E. elymoides* (PE) and three species mixture (PEB). Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

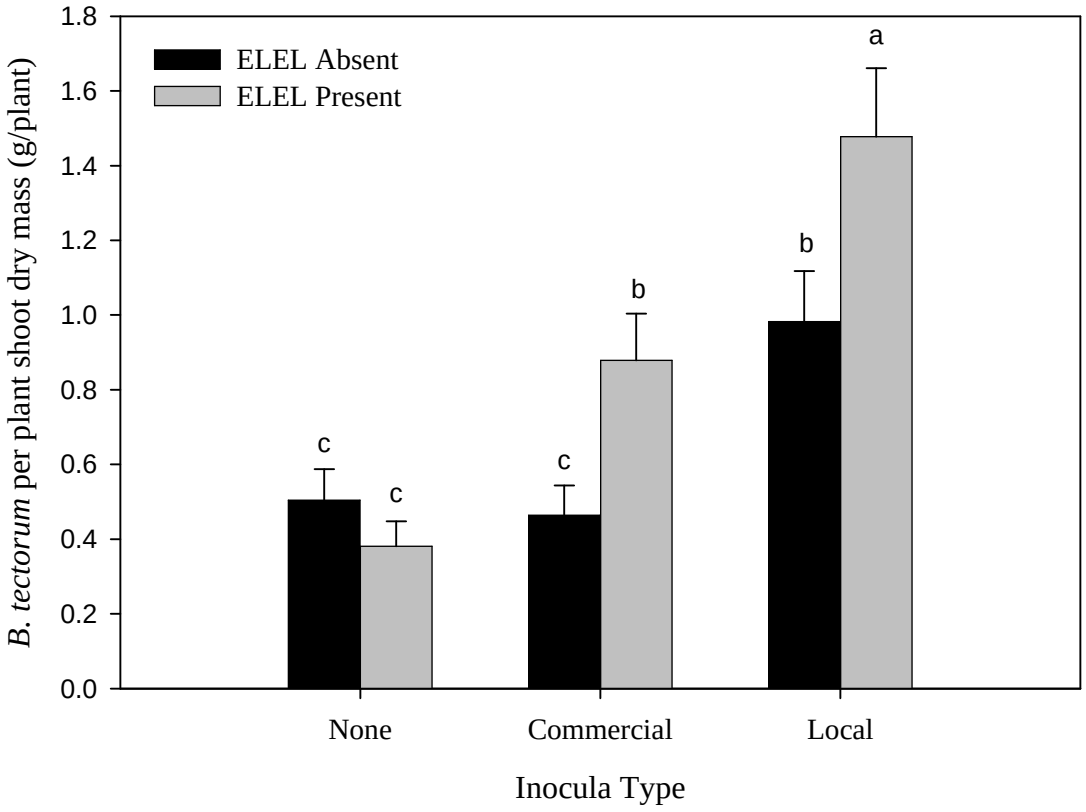

Figure 3.10 The effect of inocula type and *E. elymoides* (ELEL) competition on *B. tectorum* per plant shoot dry mass. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

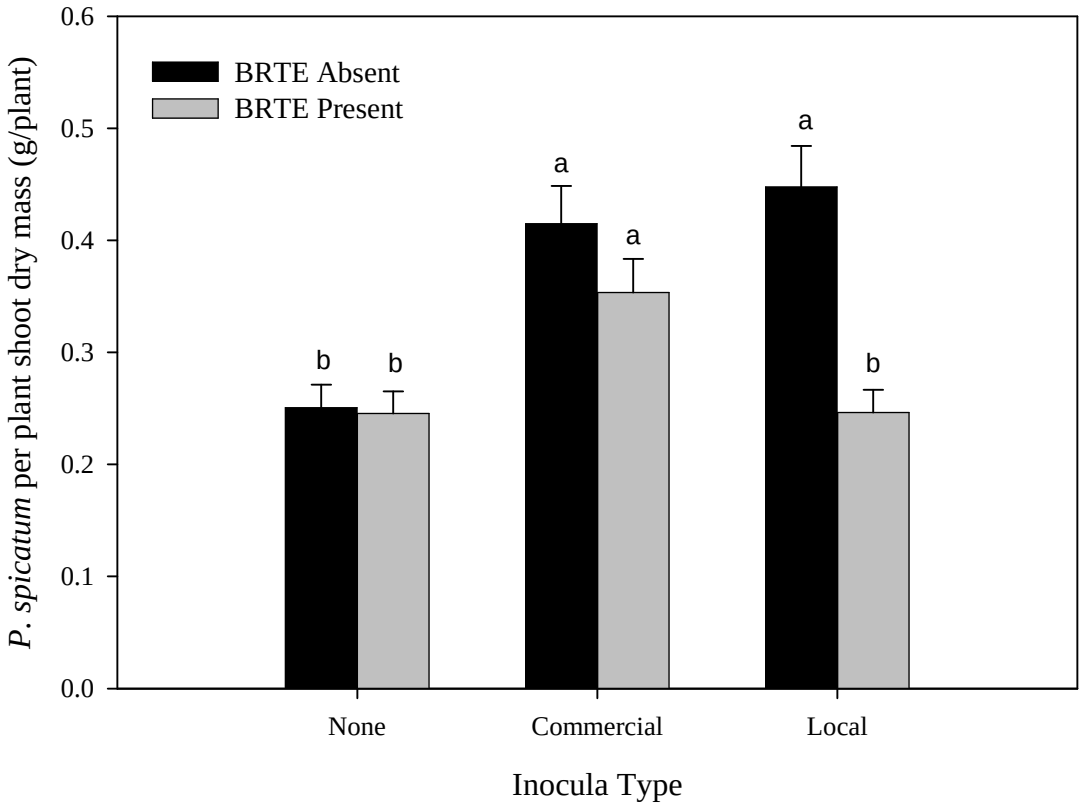

Figure 3.11 The effect of inocula type and *B. tectorum* (BRTE)competition on *P. spicatum* per plant shoot dry mass. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

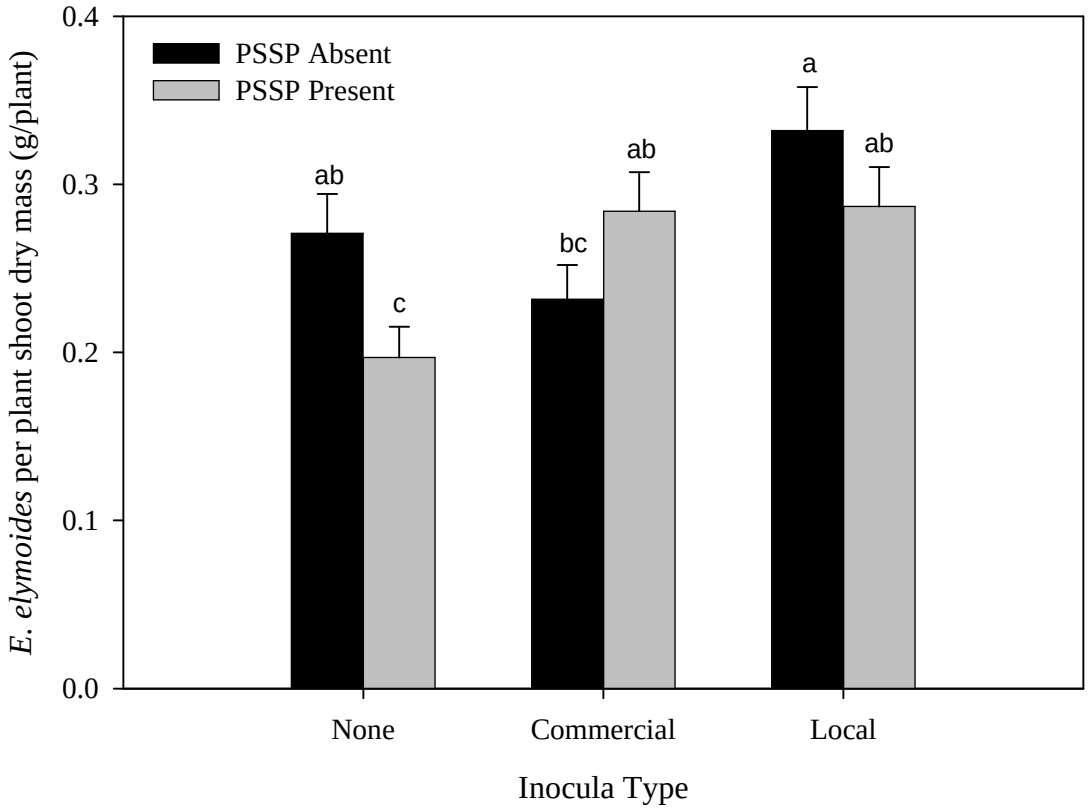

Figure 3.12 The effect of inocula type and *P. spicatum* (PSSP) competition on *E. elymoides* per plant shoot dry mass. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

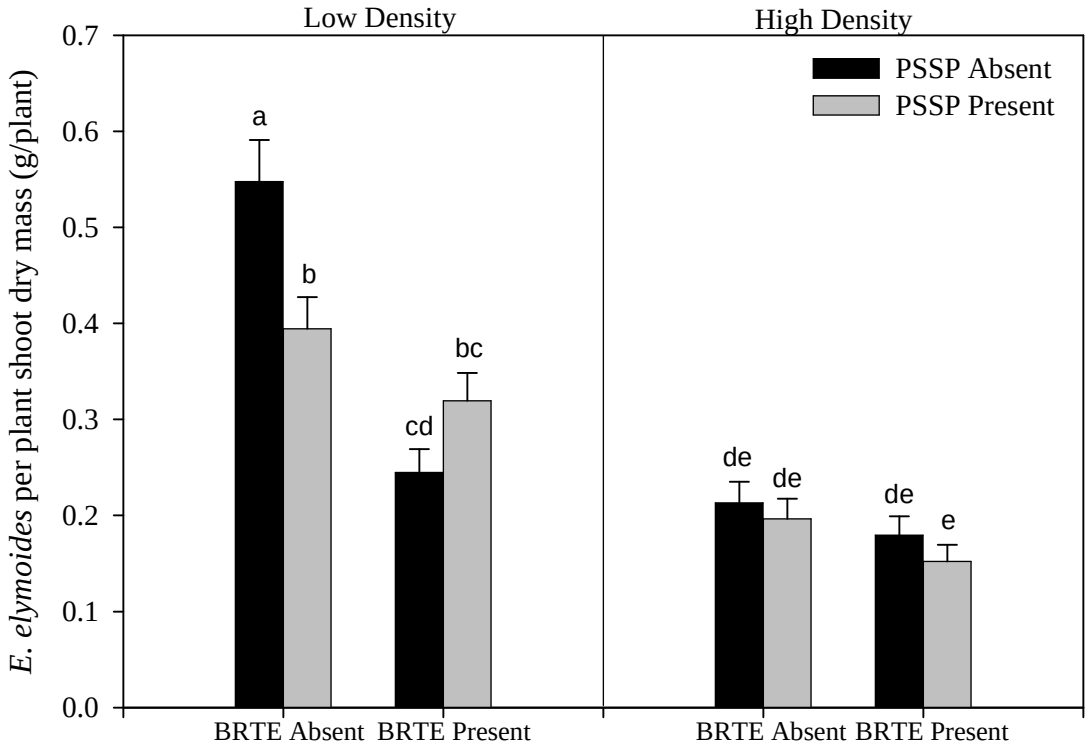

Figure 3.13 The effect of density, *B. tectorum* (BRTE) competition and *P. spicatum* (PSSP) competition on *E. elymoides* per plant shoot dry mass. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

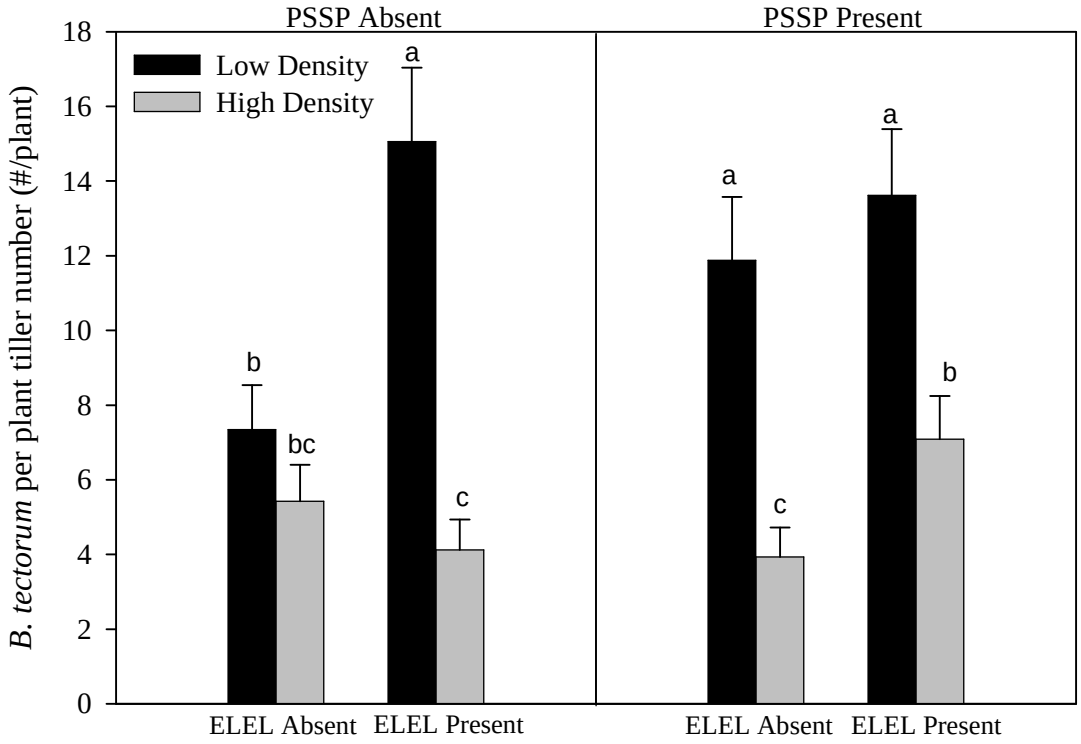

Figure 3.14 The effect of density and native perennial (*P. spicatum*, PSSP; *E. elymoides*, ELEL) competition on *B. tectorum* per plant tiller number. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

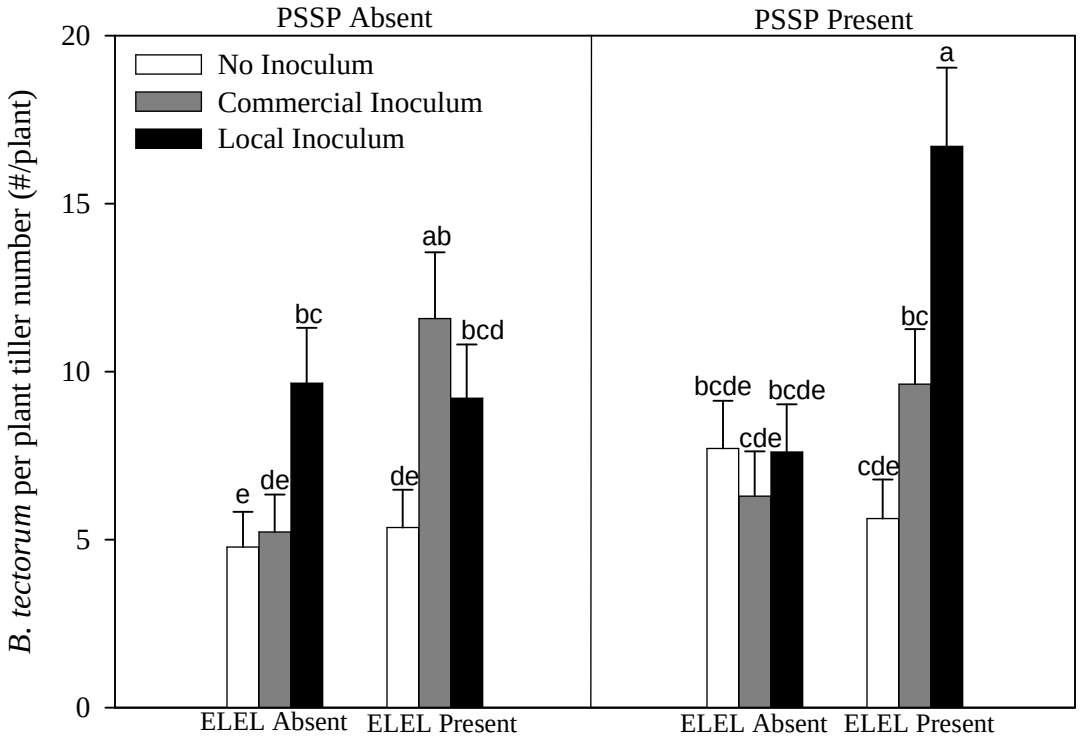

Figure 3.15 The effect of inocula type and native perennial (*P. spicatum*, PSSP; *E. elymoides* ELEL) competition on *B. tectorum* per plant tiller number. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

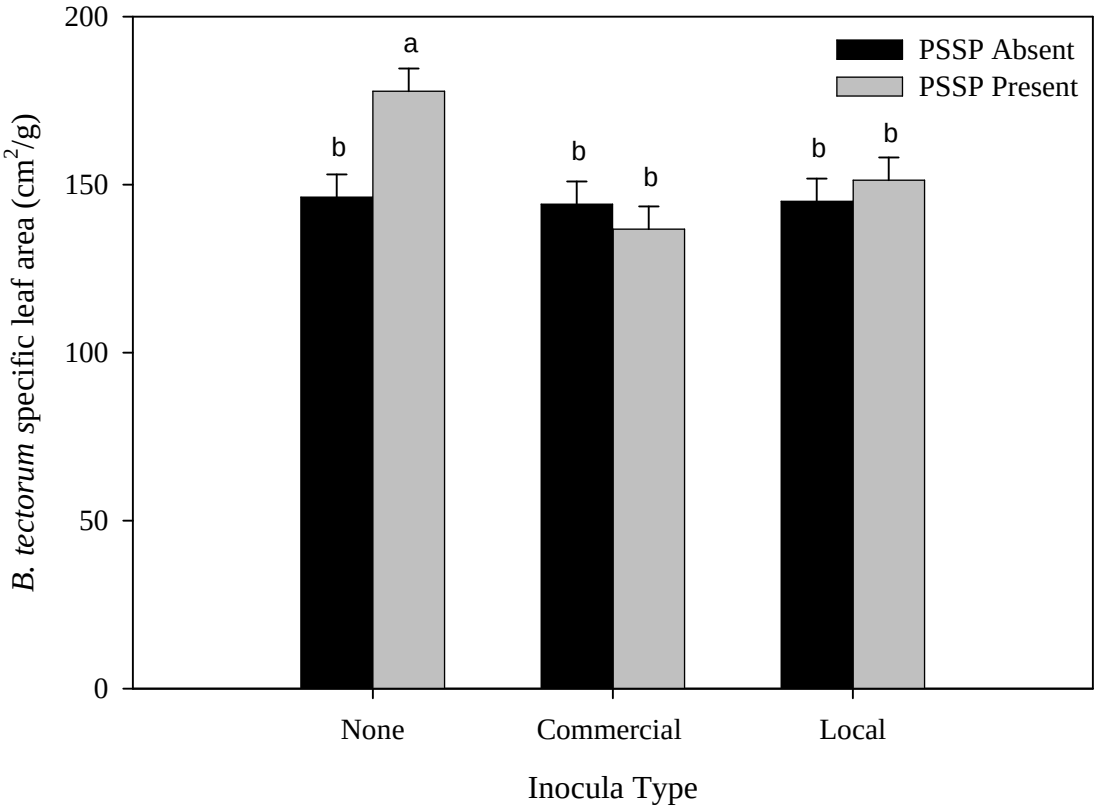

Figure 3.16 The effect of inocula type and *P. spicatum* (PSSP) competition on *B. tectorum* specific leaf area. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

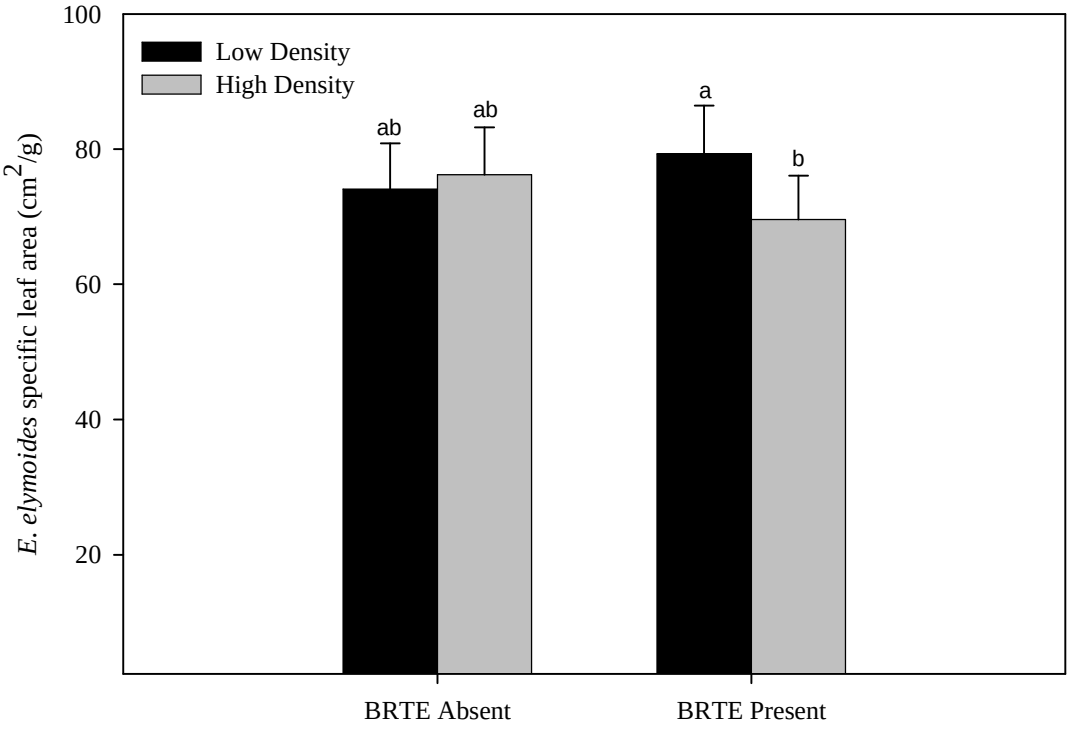

Figure 3.17 The effect of density competition and *B. tectorum* competition on *E. elymoides* specific leaf area. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

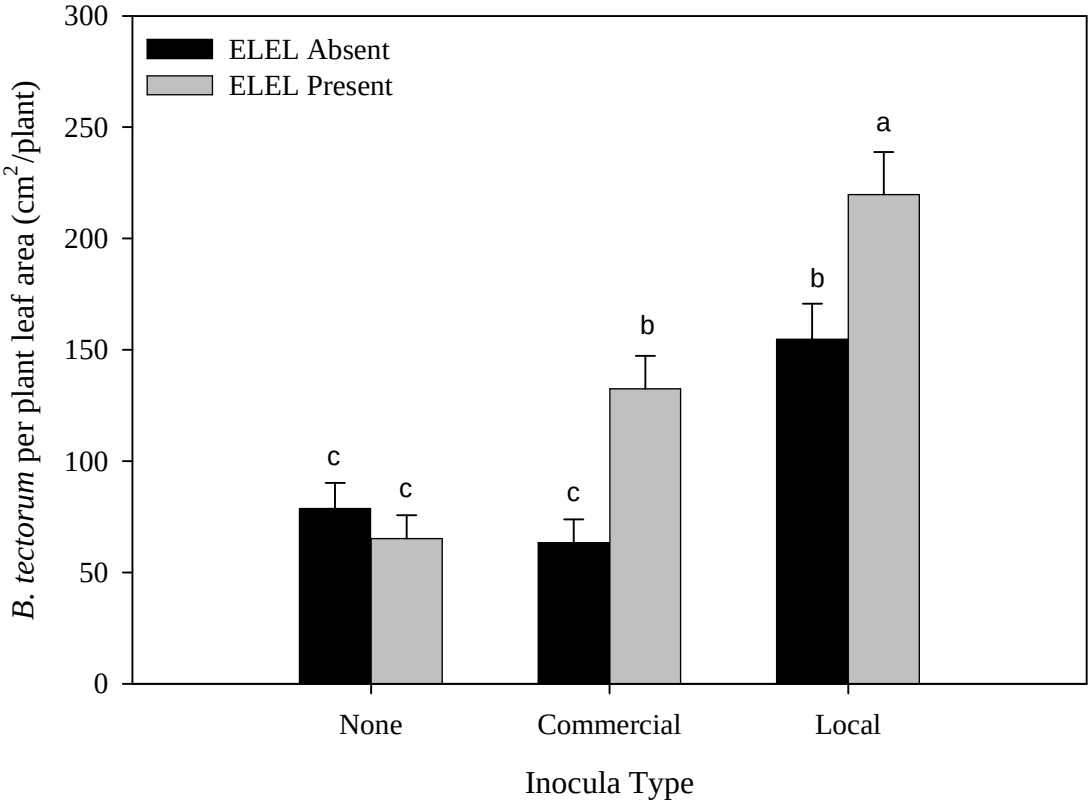

Figure 3.18 The effect of inocula type and *E. elymoides* competition on *B. tectorum* per plant leaf area. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

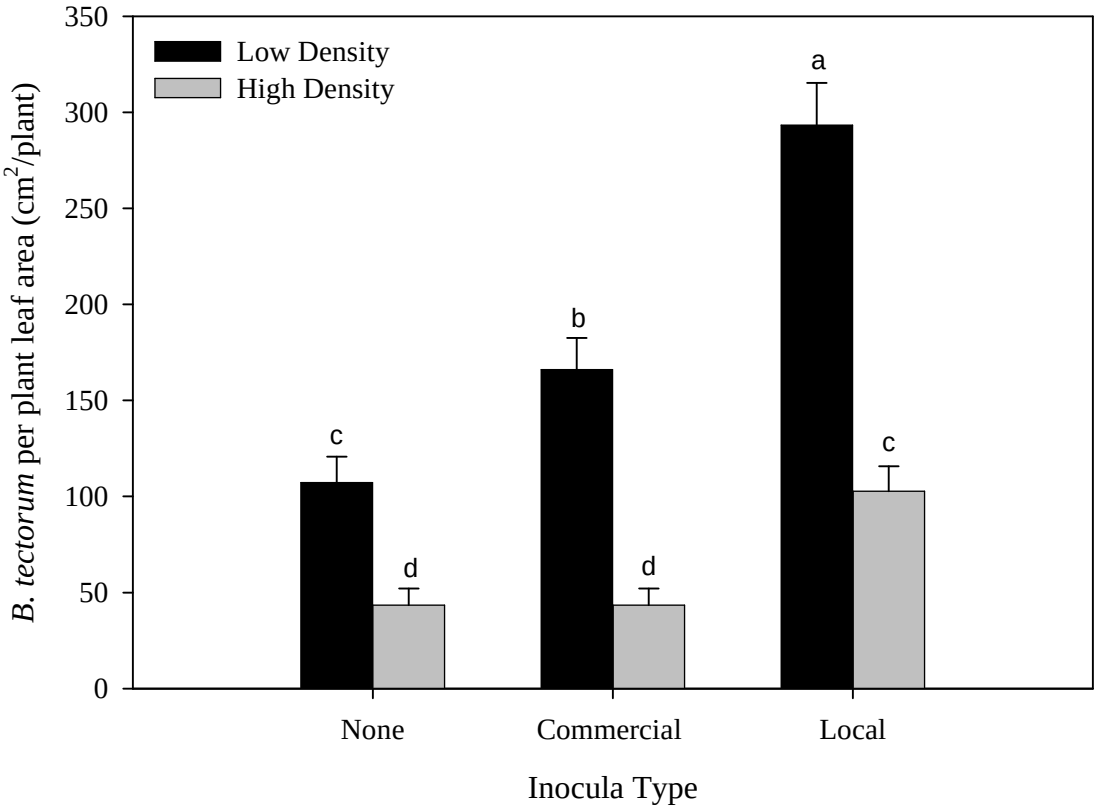

Figure 3.19 The effect of inocula type and density competition on *B. tectorum* per plant leaf area. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

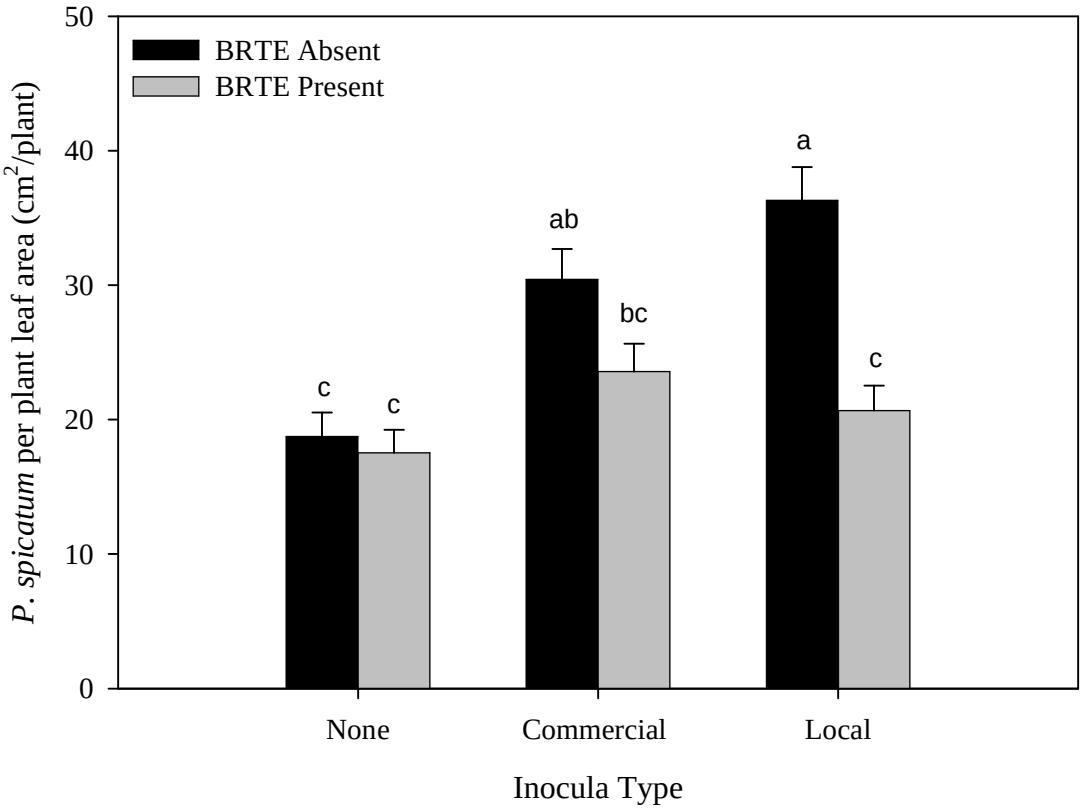

Figure 3.20 The effect of inocula type and *B. tectorum* competition on *P. spicatum* per plant leaf area. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

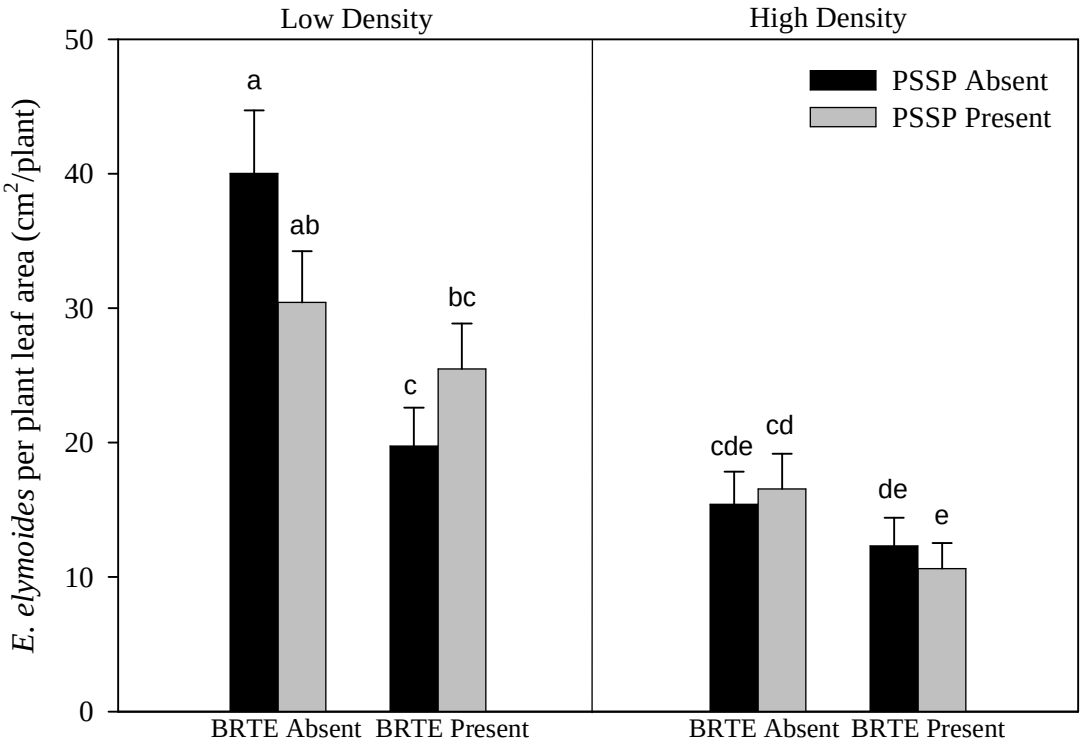

Figure 3.21 The effect of density, and interspecific (*B. tectorum*, BRTE; *P. spicatum*, PSSP) competition on *E. elymoides* per plant leaf area. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

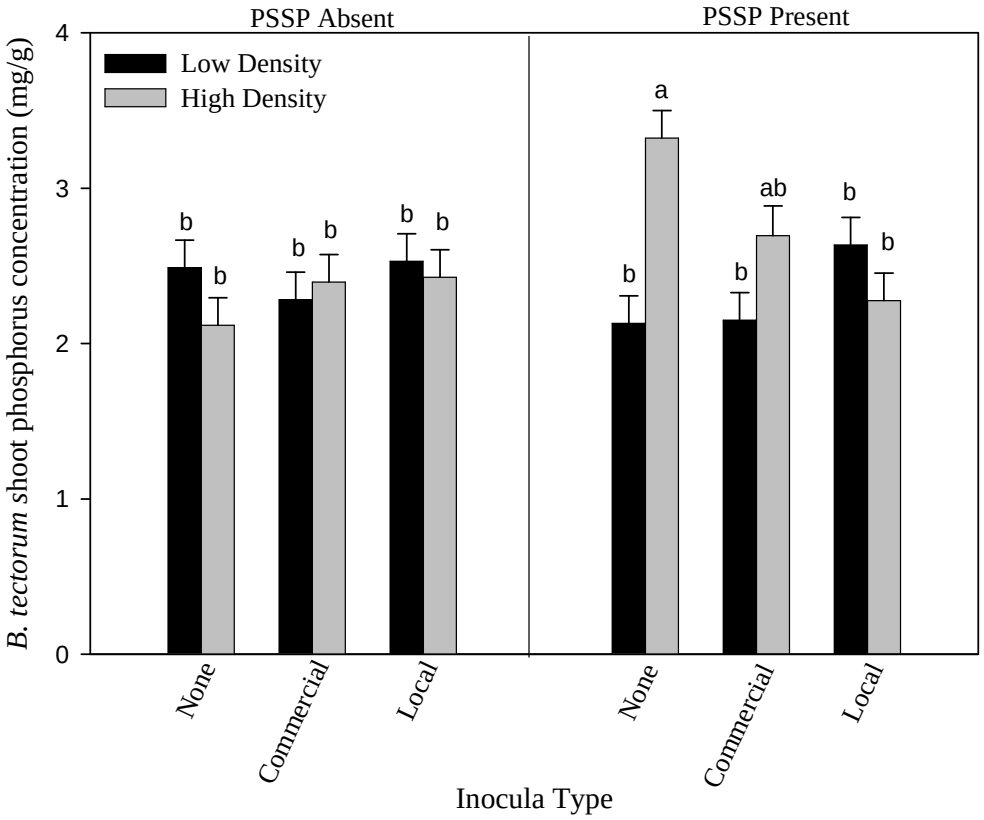

Figure 3.22 The effect of density, *P. spicatum (PSSP)* competition, and inocula type on *B. tectorum* shoot phosphorus concentration. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

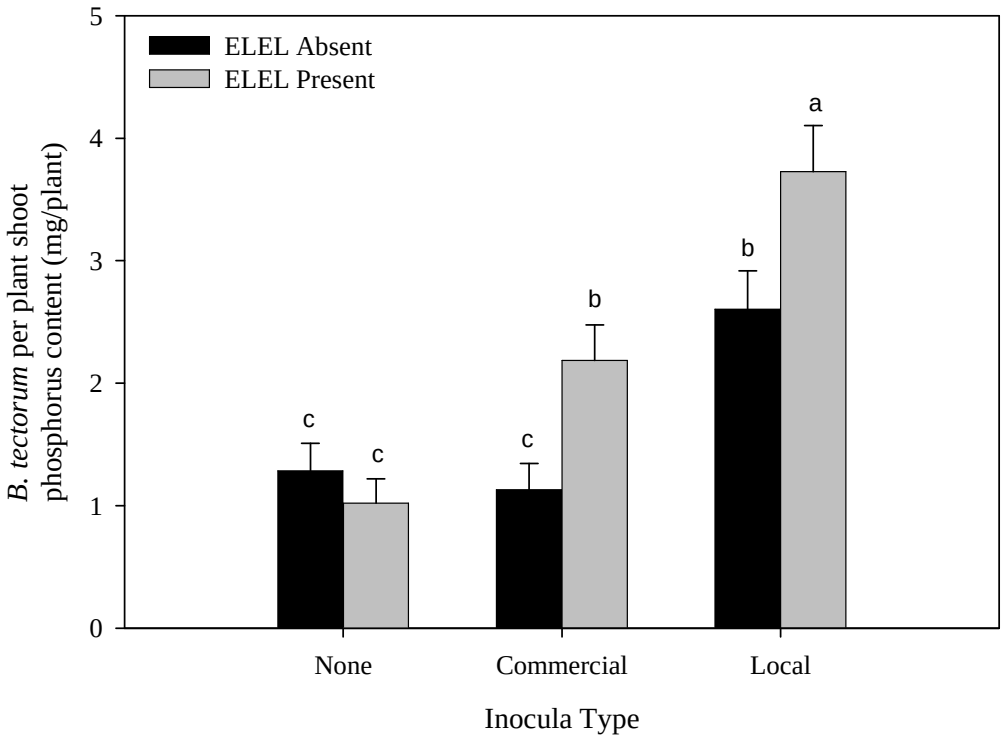

Figure 3.23 The effect of inocula type, and *E. elymoides*(ELEL) competition on *B. tectorum* per plant shoot phosphorus content. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

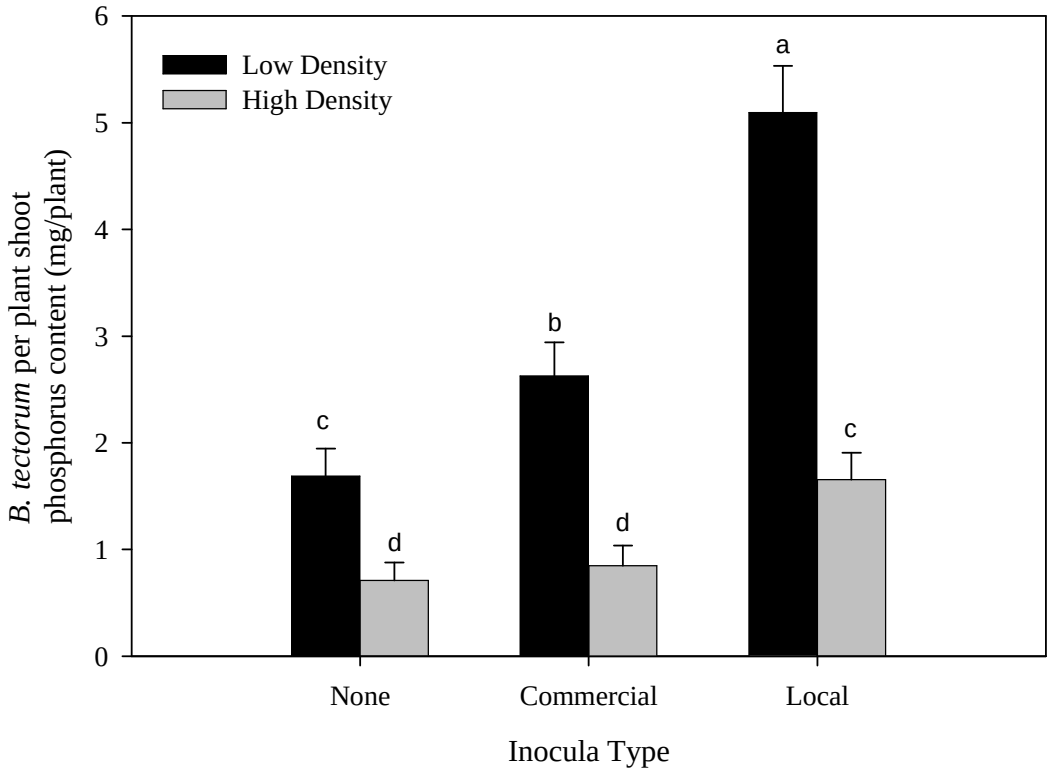

Figure 3.24 The effect of density and inocula type on *B. tectorum* per plant shoot phosphorus content. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

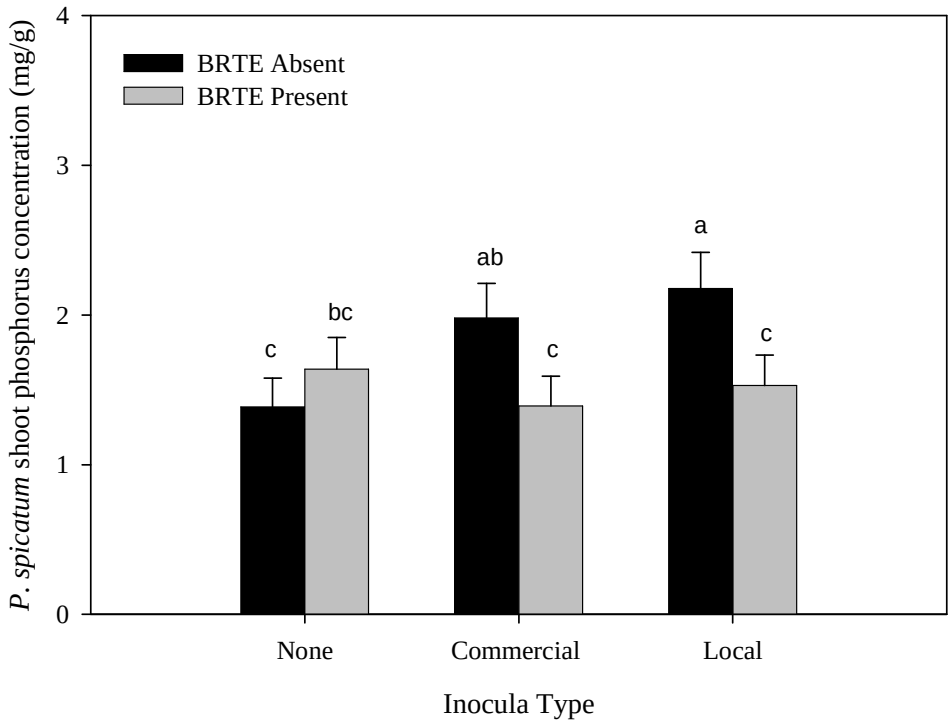

Figure 3.25 The effect of inocula type, and *B. tectorum* competition on *P. spicatum* shoot phosphorus concentrations. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

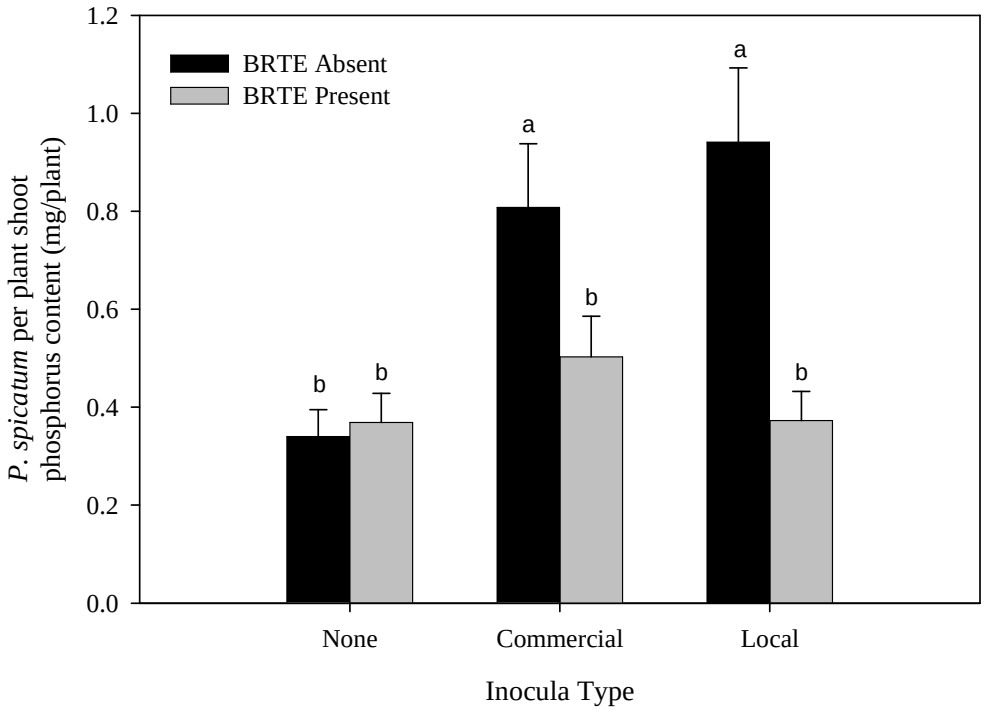

Figure 3.26 The effect of inocula type, and *B. tectorum* competition on *P. spicatum* per plant shoot phosphorus content. Bars represent means for four replicates with error bars representing + 1 standard error. Significant differences among treatment combinations are indicated by different letters ($P < 0.01$).

CHAPTER 4

MYCORRHIZAE AND RESTORATION

The introduction of *Bromus tectorum* to sagebrush communities has led to reduced native species establishment due to the *B. tectorum* – fire cycle (Stewart & Hull, 1949; Wright, 1985; Knapp, 1996; Humphrey & Schupp, 2004). As land managers and researchers have struggled to restore these highly disturbed sagebrush systems, it has become evident that alternatives to the typical seeding treatment need to be researched and developed. Since arbuscular mycorrhizal fungi (AMF) populations can be diminished in severely disturbed systems (Reeves *et al*., 1979; Allen, 1989), I have proposed that the use of AMF in restoration may help increase the competitive ability of native perennial grasses. Mycorrhizae are an important mutualism, especially in systems with low resource availability (Allen, 1996).

In chapter 2, I studied how *B. tectorum, Pseudoroegneria spicatum* and *Elymus elymoides* responded to commercial inoculum. This inoculum had a mixture of AMF species increasing the likelihood that the AMF would be compatible with the grass species. Contrary to what I expected, mycorrhizae had minimal effects on the invasive and native grass species. When mycorrhizae did have an effect it was often negative, which is not atypical for *B. tectorum* since it is not considered a mycorrhizal-dependent species. However, the negative mycorrhizal effect is atypical for *P. spicatum* and *E. elymoides*, which are considered mycorrhizal-dependent species. I concluded that the neutral and negative effects of mycorrhizae were evidence of resources being readily available, particularly P, but also water. The mycorrhizal effects in experiment 1 could also be due to the artificial conditions in my greenhouse experiment. My results illustrate

that mycorrhizal relationship range along a parasitic-mutualistic continuum depending on environmental conditions (Johnson *et al*., 1997). This highlights the importance for land managers and researchers to evaluate the abiotic status of their system before applying mycorrhizae in restoration. Mycorrhizae may not be a successful restoration tool and may be detrimental to the native species if not used wisely.

In chapter 3, I addressed how mycorrhizae altered the competitive relationship between the exotic annual grass *B. tectorum* and two native perennial grass species, *P. spicatum* and *E. elymoides*, and whether the mycorrhizal effect on competition varied with local inoculum versus commercial inoculum.

The local inoculum in general was beneficial to the perennials, but it was even more beneficial to *B. tectorum*. Some response variables such as per plant leaf area and per plant shoot P content suggested that *B. tectorum* took greater advantage of the local inoculum when competing with *E. elymoides*. *B. tectorum* per plant tiller number suggested that it took advantage of local inoculum when there was a greater proportion of native plants than of invasive plants in a pot. In contrast, in some cases, the commercial inoculum tended to be more beneficial than the local inoculum during interspecific competition for the perennials. Although plant responses varied, both inocula were beneficial to all three species.

The results described in chapter 3 demonstrate the complex dynamics of the mycorrhizal plant-fungus relationship. One particular inoculum is not necessarily always the best choice for a particular plant species. The choice of inoculum may depend on what plant physiological or morphological trait land manager and researchers consider the best indicator of competitive ability. Is greater SDM, RDM, or seed production

ultimately desired? Land managers must also take into account how the inoculum will affect the desirable species' competitors and how the desirable species mycorrhizal response will fluctuate with varying environmental condition. The question of whether or not to use mycorrhizae and what type of mycorrhizae to use does not have a simple answer due to the ever changing conditions of ecological systems. The study in chapter 3 clearly shows that inoculum can greatly benefit the non-desirable species, in some cases even more so than the desirable species.

Unless land managers are working in a static system and have thorough knowledge of their plant community's response to different AMF species, an AMF mixture is likely the best choice for inoculum (van der Heijden *et al.*, 1998). Ideally before applying inocula on a large scale project, land managers could do trial experiments to determine the desirable and non-desirable plant species responses to inocula, though given time constraints this might be difficult to achieve. Over the long-term, land managers and researchers may be able to determine the best mixture of arbuscular mycorrhizal fungal species to use for inoculum in a particular system.

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