BELOWGROUND COMPETITION AND RESPONSE TO DEFOLIATION OF *CENTAUREA MACULOSA* AND TWO NATIVE GRASSES

by

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Karla Sartor

May 2005

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ABSTRACT

Invasion of native rangelands in the western United States has serious ecological and economic effects. Understanding the mechanisms behind invasion of *Centaurea maculosa* (spotted knapweed) is necessary to effectively manage this species. Arbuscular mycorrhizae (AM), which are a type of plant fungal symbiosis, are ubiquitous in grasslands. My research explores the role of AM for increasing the competitive ability of *C. maculosa*. A greenhouse experiment tested the effects of AM fungi and neighbor species on growth of *C. maculosa*, *Festuca idahoensis* (Idaho fescue) and *Pseudoroegneria spicata* (bluebunch wheatgrass). A mesh barrier permeable to AM hyphae allowed comparison of species interactions by either roots and/or AM hyphae in pots without a barrier or by AM hyphae alone in pots with a barrier. *Centaurea maculosa* plants had high AM colonization levels within roots and ERH (extraradical hyphae), and may have increased AM colonization of neighboring plants. I found no evidence, however, that ERH affected competition, as *C. maculosa* neighbors had the greatest effect on native grass neighbors there was the potential for root contact. Additionally, plants grown with AM fungi were always smaller than non-mycorrhizal plants. In the second experiment, I investigated growth response after herbivory (simulated by clipping), with different neighboring species and AM fungi. *Centaurea maculosa*, *F. idahoensis* and *P. spicata* were grown in the greenhouse with a *C. maculosa* neighbor, with or without AM fungi, and with one of three clipping treatments (no clipping, focal plant clipped or neighbor plant clipped to remove 75% of aboveground biomass). Compensatory growth was dependent on AM fungi and neighbor species. *Centaurea maculosa* compensated for herbivory only when grown with a conspecific and with AM fungi, or with a *F. idahoensis* neighbor and no AM fungi. Clipping decreased AM colonization in *F. idahoensis* only, and colonization also decreased in *F. idahoensis* when *C. maculosa* neighbors were clipped. This research suggests that AM fungal effects vary between species in the grassland system, and is important for determining plant species response to herbivory. I also find that high levels of herbivory may reduce *C. maculosa* biomass enough to be a method for weed control, but neighbor species is important to determining plant response to herbivory.

1. INTRODUCTION

Background

Invasive plant species have serious impacts on many ecosystems. The exotic plant *Centaurea maculosa* has invaded over four million hectares of native rangelands and grasslands in western North America (Boggs & Story 1987). *Centaurea maculosa* is a perennial tap-rooted forb of the Asteraceae family. This species has very high seed production and it can overwinter as a rosette after fall germination (Watson & Renney 1974). It was first introduced from its native range of eastern Europe to western North America in the early 1900s (Watson & Renney 1974), likely originating from contaminated alfalfa. *Centaurea maculosa* invades disturbed and undisturbed lands. Dense stands of *C. maculosa* commonly occur and reduce land value because this species is not a desirable forage for domestic livestock.

Traditional methods of noxious weed control, such as herbicide application, have had only limited success. Additionally, herbicide application can be relatively expensive and some weeds may develop tolerance to commonly used herbicides (Baucom & Mauricio 2004). Alternative methods to control *C. maculosa* spread include using sheep or goat grazing, species that are known to consume both native and exotic plants (Olson & Wallander 2001). To use these tools effectively, however, a greater understanding of the factors controlling plant response to herbivory is necessary.

An arbuscular mycorrhiza (AM) is a symbiosis present in most grassland plant species (Smith & Read 1997). Soil fungi colonize the roots of plants and receive 10-20% of a plant's photosynthate (Jakobsen *et al.* 2002) in exchange for increased nutrient

acquisition for the plant. Mycorrhizal hyphae external to roots are very small in diameter, and are able to extend into soil pore spaces inaccessible to plant roots. The symbiosis is obligate for the fungal partner - they cannot grow or be cultured without the presence of a plant species - while colonization may have a beneficial or parasitic effect on the plant, depending on environmental factors (Johnson *et al.* 1997). Complex interactions can occur between plant species mediated by AM fungi. There is evidence that AM fungi can alter competitive interactions among plants through differential effects on the host plants or use of a common network of AM hyphae (Grime *et al.* 1987; Hartnett *et al.* 1993; Hartnett & Wilson 2002; Simard *et al.* 1997).

Centaurea maculosa is mycorrhizal in the field (Marler *et al.* 1999b) and AM fungi are important to interactions between this species and native rangeland species (Carey *et al.* 2004; Marler *et al.* 1999a; Zabinski *et al.* 2002). The mechanisms behind AM-induced increase in competitive ability, however, are not clear. *Centaurea maculosa* may parasitize carbon from neighboring plants (Marler *et al.* 1999a) through common mycorrhizal networks, but it was later shown that no carbon is transferred between the species (Zabinski *et al.* 2002). Instead, *C. maculosa* likely has a more efficient use of the common network of hyphae, with greater nutrient acquisition and more hyphae that extend beyond the roots (Walling & Zabinski 2004).

Plant response to herbivory is controlled by many factors, including plant growth stage at the time of defoliation (Turner *et al.* 1993), the severity of defoliation (Crawley 1997), and nutrient and water availability (Chapin & McNaughton 1989). Since AM fungi affect nutrient acquisition and availability, it can be involved with plant regrowth

after herbivory (Gehring & Whitham 1994). To predict the outcome of grazing as a weed control method, understanding the mechanisms affecting compensatory growth is necessary. Compensatory growth, defined here is growth of plants after clipping which may result in compensation for herbivory. Compensation can be defined as partial, equal or greater yield than unclipped plants (Briske & Richards 1994). Others have used the definition in terms of fruits and seeds (Maschinski & Whitham 1989) or fitness (Strauss & Agrawal 1999) in relation to unaffected plants, but in this study I will be using the term to describe biomass as an indicator of compensation.

In addition, I are interested in the effect of herbivory on AM function, because AM is a key component of grassland ecosystems. Previous literature has been mixed on whether herbivory has positive or negative effects on AM colonization. Increased colonization following herbivory may result from increased nutrient demand by the plant when plants increase growth rate to replace lost tissue. Alternatively, AM colonization may decrease in plants following defoliation since photosynthetic tissue is lower and stored carbon is allocated to regrowth instead of to AM fungal symbionts.

The aim of my first experiment (Chapter 2) was to investigate the role of AM extraradical hyphae (ERH) in the interactions between plants. In the second experiment (Chapter 3) I attempted to test a conceptual model in which the factors of neighboring species and nutrient availability regulate the ability of plants to compensate for herbivory. Both of these factors are in turn affected by AM fungi and can affect each other.

Research Objectives

My research investigated mechanisms involved in AM fungal influence on competition between *C. maculosa* and native grasses, and the role of AM fungi in the competition between these plants and their response to herbivory. The objectives of the experiment discussed in Chapter 2 were: 1) to measure the influence of arbuscular mycorrhizae and root-root interactions on competition between *C. maculosa* and two native grasses; and 2) to assess the importance of AM hyphae in plant competition when root competition is eliminated. The objectives of the experiment included in Chapter 3 were to determine: 1) whether AM influences biomass of *C. maculosa* and native grasses when grown together; 2) whether clipping alters the effect of AM fungi on host plant competitive ability; 3) whether AM fungi enhances compensatory growth in *C. maculosa* or the native grasses when grown with a neighbor; and 4) how herbivory simulated by clipping affects AM colonization, ERH density and effectiveness in increasing plant nutrient uptake and subsequent compensatory growth.

2. BELOWGROUND COMPETITION BETWEEN *CENTAUREA MACULOSA* AND TWO NATIVE GRASSES

Introduction

Centaurea maculosa is an invasive tap-rooted forb introduced to the western United States in the early 1900s. This species invades disturbed areas and intact grasslands (Tyser & Key 1988), and has invaded over four million hectares of native rangelands and grasslands in the western United States. Its establishment may pose serious ecological impacts (Watson & Renney 1974) including reduced plant diversity (Tyser & Key 1988), and economic impacts due to reduction in preferred forage plants (Watson & Renney 1974).

The invasive ability of *C. maculosa* has been attributed to a large seed production, rapid early season growth, increased ability to take up nutrients (LeJeune & Seastedt 2001), a more plastic response to N supply (Blicker *et al.* 2002), a lack of natural enemies (Keane & Crawley 2002), and allelopathic chemicals, such as (-)-catechin which may also affect the establishment and growth of neighboring plants (Bais *et al.* 2003). My research investigates the relative importance of arbuscular mycorrhizal (AM) fungi and root interactions of *C. maculosa* in competition with the native grasses *Pseudoroegneria spicata* and *Festuca idahoensis.*

Arbuscular mycorrhizae (AM) are an ancient symbiosis (Bidartondo *et al.* 2002) present in over 80% of land plants (Smith & Read 1997). The soil fungal symbiont aids plants in acquiring nutrients, while the plant symbiont donates carbon to the fungus. Arbuscular mycorrhizae can increase plant N and P (Frey & Schüepp 1993; Smith & Read 1997), which are the most limiting nutrients to plant growth (Aerts & Chapin 2000).

There is also some evidence that AM fungi provide defense against fungal pathogens (Newsham *et al.* 1995) and improve water relations (Allen & Allen 1986).

One factor influencing *C. maculosa's* ability to invade native grasslands may be its ability to compete with native plants through increased nutrient acquisition. An increased access to limiting resources or ability to grow at lower levels of resource supply often determines the outcome of plant competition (Casper & Jackson 1997; Tilman 1997). A species which benefits more than others from the AM symbiosis may be at a competitive advantage. Arbuscular mycorrhizal fungi may influence competition between species in the tall grass prairie (Hartnett *et al.* 1993; Hetrick *et al.* 1994) and in the greenhouse (Allen & Allen 1984; Fitter 1977; Marler *et al.* 1999a; Moora & Zobel 1998). Competition may be affected by AM fungi when plant species differ in the benefit they receive from mycorrhizae (Allen & Allen 1990; Bever *et al.* 2002; Grime *et al.* 1987), although that response depends on which species of AM fungi are present (Hart *et al.* 2003; Klironomos 2003).

The most important functional component of AM fungi is extraradical hyphae (ERH), the fungal mycelium external to plant roots which enable the plant to capture nutrients beyond the root's nutrient depletion zone (Fitter 1997). Extraradical hyphae can reach insoluble and immobile nutrients such as P that would otherwise be unavailable for plant uptake (Marschner 1995). *Centaurea maculosa* produces more extraradical hyphae than *F. idahoensis* (Walling & Zabinski 2004), which could confer a competitive advantage to *C. maculosa* if it can access more limiting nutrients than native grasses.

Marler *et al*. (1999a) found that the negative effect of *C. maculosa* on the biomass of a *F. idahoensis* neighbor was enhanced by the presence of AM fungi.

The objectives of this experiment were: 1) to measure the influence of arbuscular mycorrhizae and root-root interactions on competition between *C. maculosa* and two native grasses; and 2) to assess the importance of AM hyphae in plant competition when root competition is eliminated.

Methods

Experimental Setup

This experiment was a randomized complete factorial design, including nine species combinations, two barrier treatments, two AM treatments, and nine replicates of all treatments. *Festuca idahoensis, P. spicata* and *C. maculosa* were grown in inter- and intra-specific combinations and alone. Each species was seeded directly into 7500 ml pots and later randomly thinned to one seedling. *Festuca idahoensis* was seeded nine days prior to seeding *P. spicata* or *C. maculosa* because of its longer germination and establishment time. Seedlings started at the time of direct seeding were transplanted into pots without establishment.

All species combinations were grown either with no barrier or in pots with a 28 μm mesh barrier (Nitex™ nylon, Sefar America, Depew, NY, USA) bisecting the pot. The barrier excluded roots but not AM hyphae, and has minimal effect on water and solute movement (Zimmerley, unpublished data). In pots with two plants, the barrier excluded neighbors' roots but not hyphae, and in pots with a single plant, the barriers created a compartment that only hyphae could access.

Plants in all treatments were grown under either AM or non-AM conditions. Unpasteurized field soil served as a mycorrhizal inoculum for AM treatment plants, while for non-AM treatments the entire soil mix was aerated steam pasteurized at 80 °C for 90 minutes. To reintroduce non-AM microbes, 800 ml of field soil was mixed with 4000 ml of DI water and vacuum-filtered to pass through an 11μm filter (Whatman No. 1) three times. Ten ml of microbial wash was added to each side of all pots in the non-AM treatment.

Plants were grown for 16 weeks under greenhouse conditions maintained at a 21 ºC daytime and 16 ºC nighttime temperature with supplemental lighting (GE Multi-Vapor MVR1000/C/U) as necessary for a 16-hour day length. Plants were grown in 4:1 sand:soil mix, with 30 grit silica sand and a 1:1 mix of pasteurized loam top soil and field soil. Field soil was collected adjacent to the Red Bluff Research Ranch near Norris, Montana (45.60°, 111.50°) in an *F. idahoensis, P. spicata* dominated grassland with *C. maculosa* present, and sieved through a 1.5cm screen. The soil mixture had initial NO_3 and P levels of 4.9mg/kg and 3.3mg/kg, respectively. I added 50ml of 1/2 strength Hoagland's solution to each side of the pot two times during the experiment as plants showed signs of deficiency.

Plant Analysis

Root and shoot tissue was separated, cleaned and dried until weight did not change, and biomass was recorded. Three replicates of plants were randomly chosen from each treatment for each species to be analyzed for tissue nutrient analysis. Root and shoot tissues were combined and ground to pass through a 0.5μm screen (UDY mill

Model #3010-030; Fort Collins, CO, USA). Kjeldahl digestion for N and nitric acid/hydrogen peroxide digestion for P analysis were performed, and then analyzed using Inductively Coupled Plasma Spectrometry (MDS Harris, Lincoln, NE, USA).

Mycorrhizal Analysis

To determine colonization levels in mycorrhizal and non-mycorrhizal treatments, fine root samples of five randomly selected replicates of plants from AM treatments and two replicates of non-AM treatments were cleared for 48 hours in 2.5% KOH, rinsed with distilled water, acidified for 12 hours with 3% HCL, and stained for 12 hours with 0.05% Trypan blue solution (Phillips & Hayman 1970). Mycorrhizal structures including internal hyphae, vesicles and arbuscules were quantified using the magnified line intersect method (McGonigle *et al.* 1990).

Extraradical hyphae were quantified from two 2.5 cm diameter soil cores extracted from each pot. For pots with two plants, cores were 3 cm from the base of each plant. Pots with only one plant were cored 3 cm from the plant and again in the center of the hyphal compartment. Extraradical hyphae were extracted from these samples using a modified method of Miller *et al.* (1995). Soil cores were mixed well, and two replicate 5g samples were mixed with 15 ml of 3.75% sodium hexametaphosphate and 100ml water, stirred for 5 minutes, and allowed to sit for 30 minutes to dissociate soil particles. An additional sample of each soil was dried to obtain dry weight equivalents. A 10ml aliquot was diluted with 100ml water, and 20ml was removed and filtered through a 20µm mesh filter. The filter was placed in a centrifuge tube and stained with 5 ml 0.5% Trypan blue for 5 minutes. The contents were filtered through a 0.45µm membrane filter.

AM hyphae were distinguished from non-AM hyphae by characteristics such as diameter, the presence of angular projections and a general lack of septae (Mosse 1959; Nicolson 1959; Sylvia 1992). The length of AM hyphae on each filter was estimated using a grid line intersect method (Tennant 1975) under 200x magnification and used to calculate length of hyphae g^{-1} soil.

Statistical Analysis

Variables including root, shoot and total biomass, root mass ratio (calculated as the ratio of root biomass:total biomass), AM percent colonization, ERH density, root length, and N and P concentration and content were analyzed by ANOVA using R 2.0.1 (R Development Core Team 2004). Three–way ANOVA's for the factors of AM (+/-), barrier (+/-), and neighbor species (*C. maculosa, F. idahoensis, P. spicata* or no neighbor), and all interactions were analyzed for each variable individually for each plant species. For ERH measurements, contrasts were used to identify ERH density greater than the mean background density for non-AM pots. Power transformations were identified using the Box-Cox procedure and homogeneity of variance was verified with the modified Levene's test. Tukey's multiple comparison procedure was used to separate means ($\alpha = 0.05$).

Results

Biomass

Centaurea maculosa plants had a biomass of 5.9g ± 0.2g, while *P. spicata* and *F. idahoensis* were 4.9g ± 0.2g and 0.9g ± 0.1g respectively. The AM treatment had a

significant effect on biomass (Table 1) – plants were 46%, 50% and 39% smaller grown under AM than non-AM conditions for *C. maculosa*, *F. idahoensis* and *P. spicata* plants, respectively (Figure 1a).

Table 1. Results of ANOVA for total biomass. *C. maculosa* and *F. idahoensis* were log transformed and *P. spicata* was square root transformed.

		C. maculosa		*F. idahoensis*		*P. spicata*	
	Residual df:	127		124		127	
Factor	df	F	p	F	p	F	p
AM	1	172.36	0.000	27.24	0.000	99.94	0.000
Neighbor	3	35.44	0.000	14.55	0.000	53.25	0.000
Barrier	1	2.84	0.094	12.09	0.001	2.59	0.110
AM X Neighbor	3	0.43	0.733	1.10	0.353	1.10	0.351
AM X Barrier	1	2.78	0.098	0.13	0.715	1.36	0.246
Neighbor X Barrier	3	1.22	0.306	3.76	0.013	10.27	0.000
AM X Barrier X Neighbor	3	0.52	0.669	0.93	0.430	0.89	0.447

Total biomass of all species was affected by neighbor (Table 1). *Centaurea maculosa* biomass was smallest with *C. maculosa* and *P. spicata* neighbors and largest with either *F. idahoensis* or no neighbor (Figure 1b). For *F. idahoensis*, total biomass was smallest with a *C. maculosa* neighbor and largest with either *F. idahoensis* neighbor or when growing alone. *Festuca idahoensis* and *P. spicata* neighbors affected *F. idahoensis* biomass similarly (Figure 1b). *Pseudoroegneria spicata* was largest growing alone, and smallest growing with a *C. maculosa* neighbor, and was also smaller growing with a *P. spicata* neighbor than a *F. idahoensis* neighbor (Figure 1b).

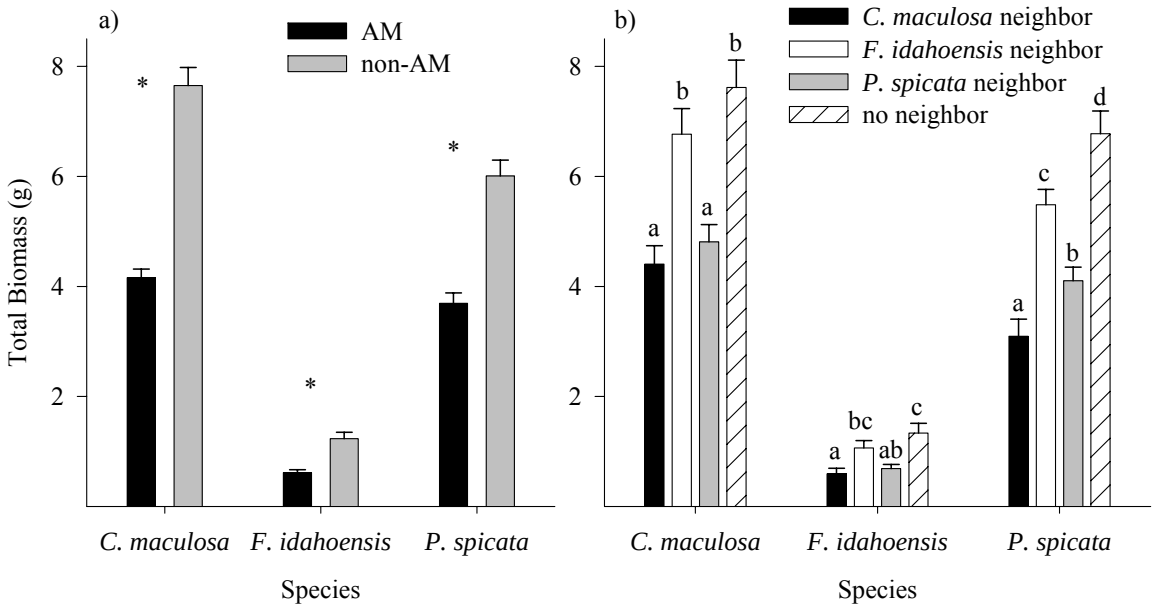

Figure 1. Total biomass (g) of each species by (a) AM and (b) neighbor treatments. Error bars represent one standard error of the mean. (*) Indicates significant difference between means of AM vs. non-AM treatments ($\alpha<0.05$). Letters represent significant differences between treatments within each species ($\alpha<0.05$).

Biomass of *F. idahoensis* and *P. spicata* varied by barrier and neighbor factors (Table 1). Both *F. idahoensis* and *P. spicata* plants were larger when a barrier separated them from a *C. maculosa* neighbor (Figure 2). For *F. idahoensis,* the barrier eliminated competition from all other neighboring species, in that plants separated from their neighbor by a barrier were the same size as plants grown with no neighbor. Competition occurred with the barrier present for inter- and intraspecific competition of *C. maculosa* and *P. spicata*. Both species were larger growing alone than grown with a *C. maculosa* or *P. spicata* neighbor, even with a barrier present (Figure 2).

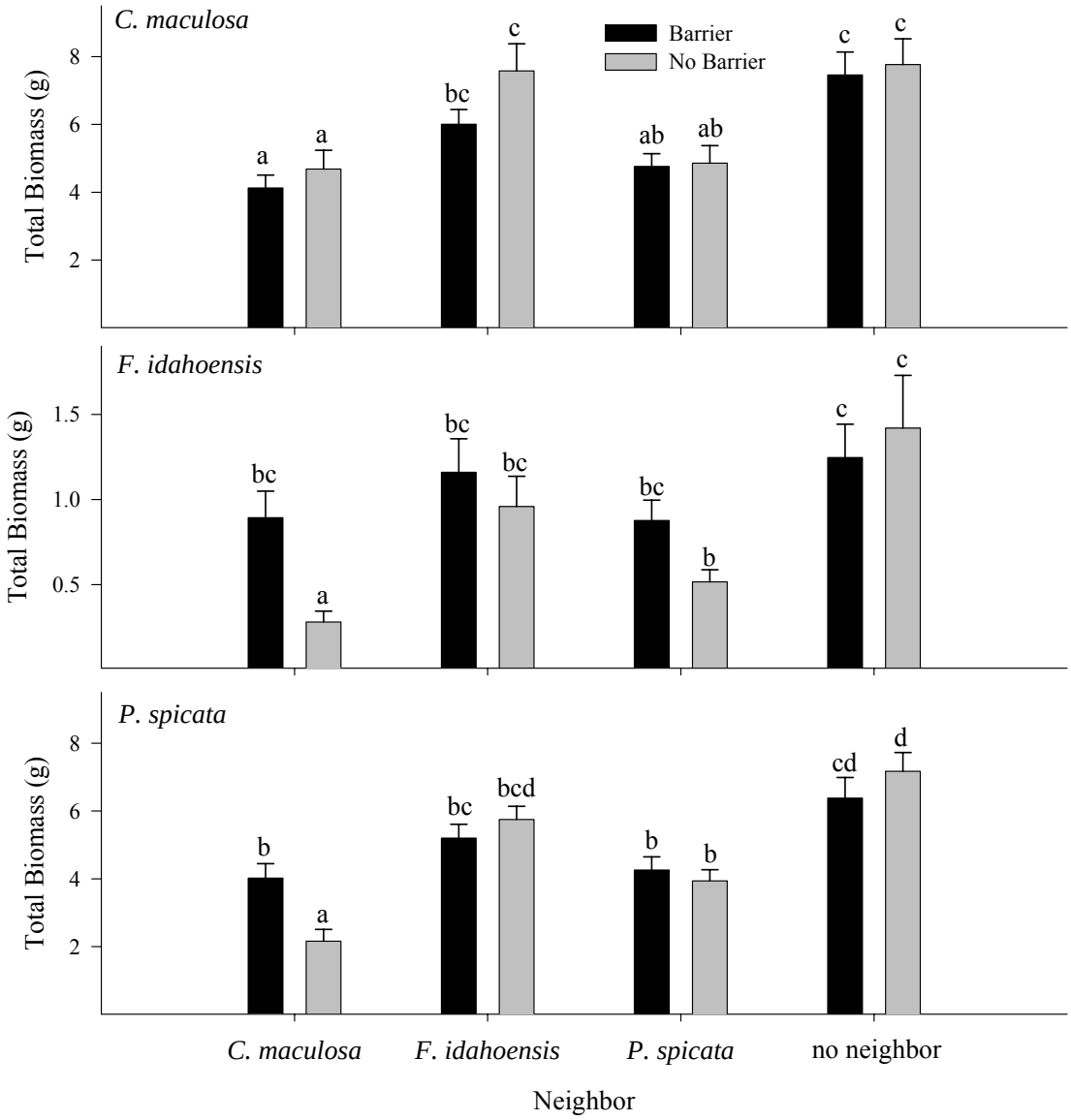

Figure 2. Neighbor and barrier effects on total biomass for each species. Note difference in y-axis scales. Error bars represent one standard error of the mean. Letters represent significant differences between treatments within each species ($\alpha<0.05$).

Root Mass Ratio

Root mass ratios were 0.62, 0.46, and 0.54, for *C. maculosa*, *F. idahoensis* and *P. spicata,* respectively. Root mass ratios were lower in the AM treatment for *C. maculosa* and *F. idahoensis* (Table 2; Figure 3a). The barrier treatment only affected *C. maculosa* (Table 2), with higher RMR in pots with a barrier (Figure 3a).

Table 2. Results of ANOVA for RMR. No transformations were performed.

	C. maculosa			*F. idahoensis*		*P. spicata*	
	Residual df:	127		124		127	
Factor	df	F	p	F	p	F	p
AM	1	3.69	0.057	13.48	0.000	0.40	0.526
Neighbor	3	4.27	0.007	10.53	0.000	1.80	0.151
Barrier	1	8.06	0.005	0.18	0.669	0.04	0.834
AM X Neighbor	3	1.67	0.177	1.53	0.210	2.72	0.047
AM X Barrier	1	3.32	0.071	1.01	0.317	0.20	0.657
Neighbor X Barrier	3	0.17	0.918	4.25	0.007	1.88	0.137
AM X Barrier X Neighbor	3	0.59	0.621	0.27	0.847	0.26	0.854

Neighbor species affected RMR for both *C. maculosa* and *F. idahoensis* (Table 2). Both species had higher RMR when grown with the other species. *Centaurea maculosa* had higher RMR grown with a *F. idahoensis* neighbor and *F. idahoensis* had higher RMR grown with a *C. maculosa* neighbor than grown with a conspecific (Figure 4). The effect was only apparent for *F. idahoensis* without the barrier (Table 2, data not shown).

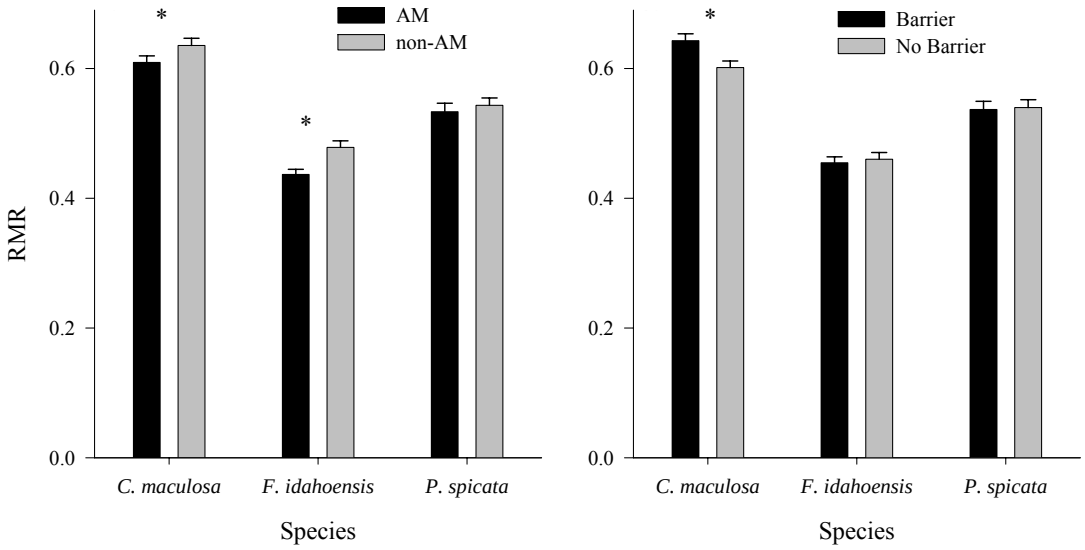

Figure 3. AM effects and barrier effects on RMR for each species grown with all neighbors. Error bars represent one standard error of the mean. (*) Indicates significant difference between means of AM vs. non-AM treatments ($\alpha<0.05$).

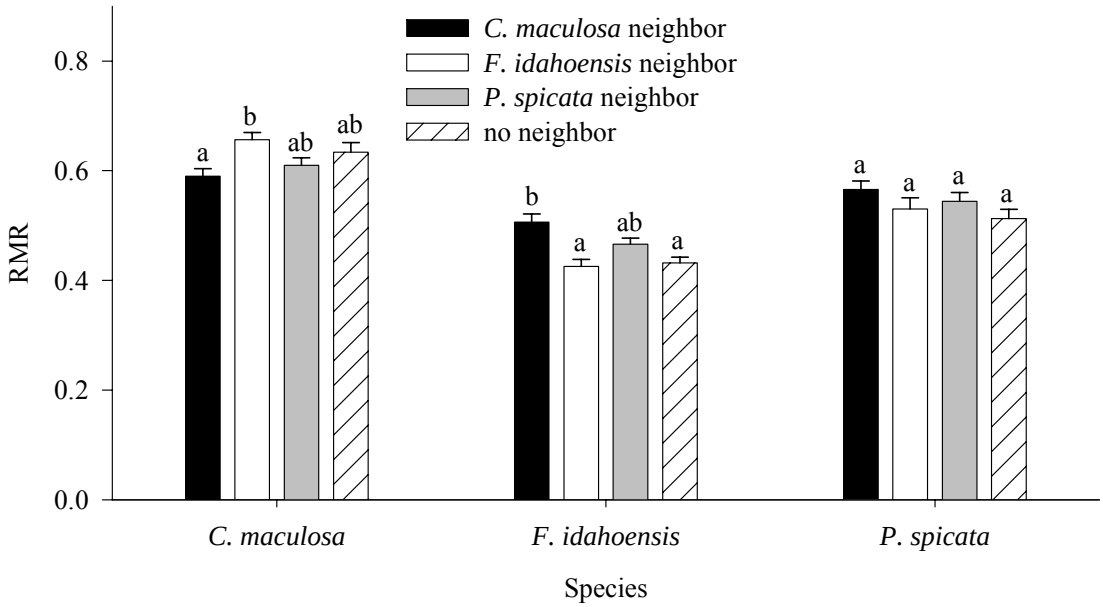

Figure 4. Neighbor effects on root mass ratio (RMR) of each species. Error bars represent one standard error of the mean. Letters represent significant differences between treatments within each species ($\alpha<0.05$).

AM Colonization

Percent of root intersections colonized by AM fungi was highest for *C. maculosa* (18.3 ± 1.9%) and lowest for *F. idahoensis* and *P. spicata* (3.3 ± 0.7% and 7.0 ± 1.0%). Only one intersection in one plant in the non-AM treatment had traits similar to AM hyphae (mean <0.010% colonization), and no vesicles or arbuscules were present in the non-AM treatment roots.

Table 3. Results of ANOVA for AM colonization % in AM treatment pots. *C. maculosa* and *P. spicata* were not transformed and *F. idahoensis* was log transformed plus one.

		C. maculosa		*F. idahoensis*		*P. spicata*	
	Residual df:	44		48		43	
Factor	df	F	p	F	p	F	p
Neighbor	3	0.81	0.495	14.25	0.000	2.43	0.078
Barrier	1	5.28	0.026	1.53	0.223	0.06	0.813
Neighbor X Barrier	3	1.52	0.224	2.12	0.110	0.25	0.858

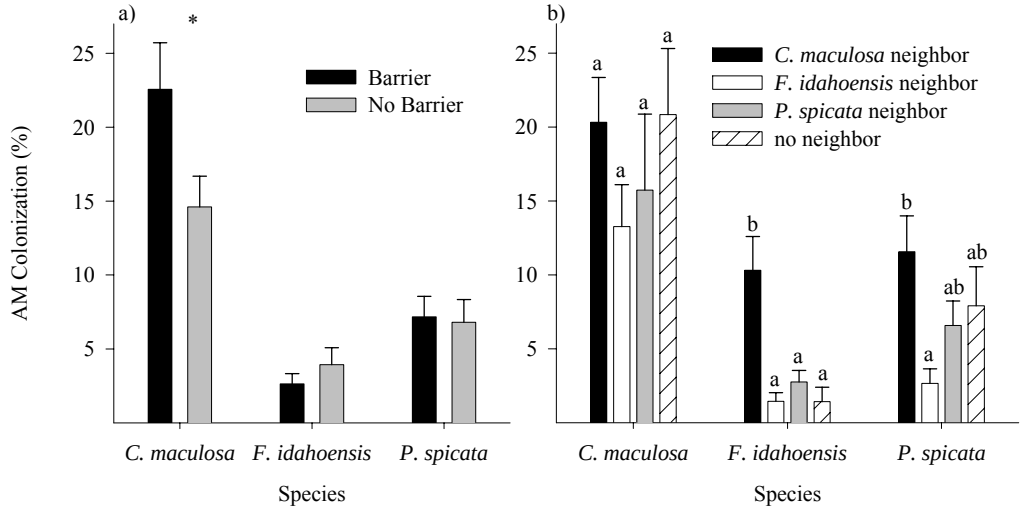

Figure 5. AM colonization (%) by (a) barrier and (b) neighbor treatments. Error bars represent one standard error of the mean. (*) Indicates significant difference between means of AM vs. non-AM treatments (α<0.05). Letters represent significant differences between treatments within each species (α<0.05).

The barrier treatment affected colonization levels for only *C. maculosa* (Table 3). In pots with a barrier, *C. maculosa* had 23% of intersections colonized, while pots without a barrier had 15% colonization (Figure 5a). Neighbor identity affected percent root length colonized in *F. idahoensis* (Table 3). Mean colonization levels in *F. idahoensis* were 10.3% grown with a *C. maculosa* neighbor and only 1.4 – 2.8% with each grass species neighbor and growing alone (Figure 5b). Neighbor species influenced *P. spicata* (p=0.078), and colonization levels were higher with *C. maculosa* neighbors than with *F. idahoensis* neighbors (Figure 5b). Neighbor identity did not affect *C. maculosa* AM colonization (Table 3).

Extraradical Hyphae

A background ERH density of 1.88 ± 0.6m g^{-1} soil was present in non-AM pots, likely due to non-viable hyphae in pasteurized soil inoculum added to the non-AM soil medium. Extraradical hyphal density exceeded the background level only in *C. maculosa* pots and *F. idahoensis* pots with a *C. maculosa* neighbor (Figure 6), determined by contrasts. Hyphal density (m g^{-1} soil) in soil extracted from the *C. maculosa* side of the pot was not affected by neighbor species or barrier treatment (Table 4). For the *F. idahoensis* side of the pot, neighbor species affected hyphal density (Table 4). Hyphal density exceeded background levels only in pots with a *C. maculosa* neighbor. *Pseudoroegneria spicata* pots did not have ERH density greater than non-AM pots for any treatment combination (Figure 6). Extraradical hyphal density did not vary by neighbor and barrier treatments. Extraradical hyphal density did not vary by and interaction of neighbor and barrier treatments.

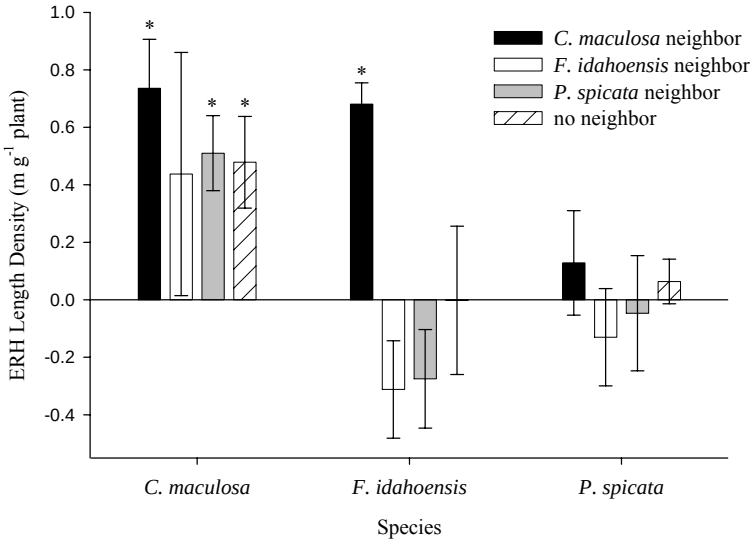

Figure 6. Difference in extraradical hyphae length density for AM treatments vs. mean of non-AM treatments (background level). Error bars represent one standard error of the mean. (*) represents significant hyphal length density above background levels ($\alpha<0.05$).

For *C. maculosa* plants with no neighbor, samples taken on the opposite side of the barrier (hyphal compartment) did not differ in ERH density from samples on the plant side (Table 5), indicating that plants were able to access resources in the hyphal compartment through ERH. Extraradical hyphal density in the hyphal compartment did not differ from that in the distant side of pot (Table 5). Pots with grass species grown alone did not have significant hyphal density above the background density, so the side of pot analysis was not performed.

Table 4. Results of ANOVA for extraradical hyphal length density.

		C. maculosa		*F. idahoensis*		*P. spicata*	
	Residual df:	22		22		23	
Factor	df	F	p	F	p	F	p
Intercept	1	325.91	0.000	543.11	0.000	534.57	0.000
Neighbor	3	0.02	0.996	7.96	0.001	1.70	0.194
Barrier	1	0.39	0.536	2.50	0.128	0.01	0.922
Neighbor X Barrier	3	0.98	0.420	0.10	0.961	1.93	0.153

Table 5. Results of ANOVA for *C. maculosa* extraradical hyphal length density when plants were grown alone.

Factor	df	F	p
Barrier	1	0.01	0.927
Side of pot	1	0.01	0.934
Barrier X Side of pot	1	0.77	0.407
Residual	8		

Plant Nitrogen

Plant tissue percent N was 1.41 ± 0.04% in *F. idahoensis*, 0.92 ± 0.06% in *P. spicata* and 1.06 ± 0.05% in *C. maculosa*. The AM treatment affected nitrogen concentration for *C. maculosa* and marginally for *F. idahoensis* (Table 6). Nitrogen concentration in *C. maculosa* was higher under AM conditions, and the effect was strongest without a barrier (Figure 7). *Festuca idahoensis* also had a trend toward higher N concentration under AM conditions, but was not affected by the barrier (Figure 7).

Table 6. Results of ANOVA for tissue percent N. No transformations were performed.

	C. maculosa			*F. idahoensis*		*P. spicata*	
	Residual df:	27		34		33	
Factor	df	F	p	F	p	F	p
AM	1	6.90	0.013	3.81	0.061	1.15	0.292
Neighbor	3	3.31	0.032	6.27	0.002	0.99	0.410
Barrier	1	5.68	0.023	0.41	0.525	2.98	0.094
AM X Neighbor	3	0.95	0.430	0.57	0.641	1.74	0.177
AM X Barrier	1	7.78	0.009	0.12	0.730	1.31	0.261
Neighbor X Barrier	3	0.75	0.531	0.64	0.595	0.63	0.603
AM X Neighbor X Barrier	3	0.08	0.972	0.74	0.540	1.53	0.224

Neighbor species affected N concentration in *C. maculosa* and *F. idahoensis* (Table 6). For *C. maculosa*, N concentration was highest with a *P. spicata* neighbor while *F. idahoensis* tissue N concentration was highest with a conspecific, or growing

alone, and lowest with a *C. maculosa* neighbor (Figure 8). *Pseudoroegneria spicata* tissue N did not vary between treatments.

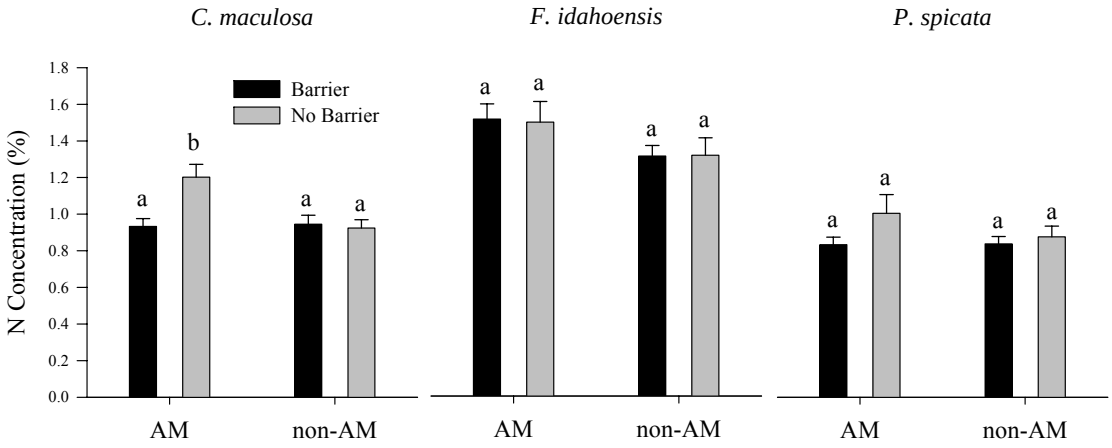

Figure 7. N concentration (%) with am and barrier treatments. Error bars represent one standard error of the mean. Letters represent significant differences between treatments within each species ($\alpha<0.05$).

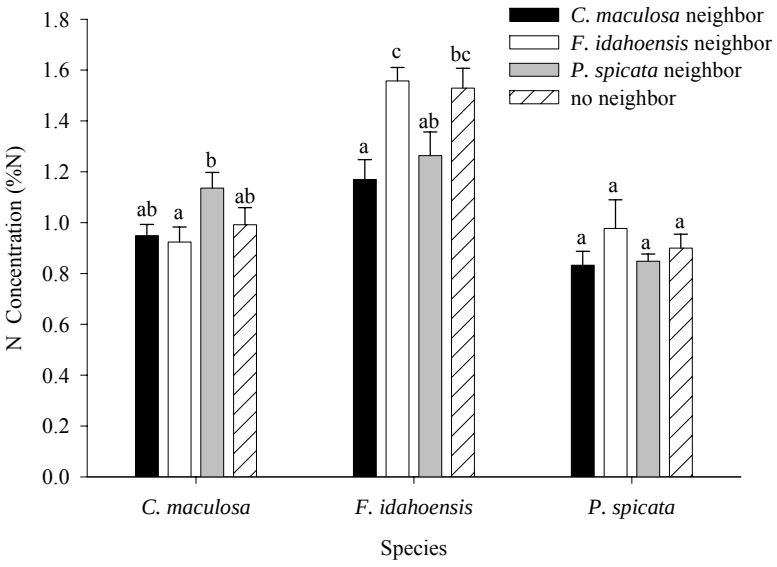

Figure 8. Nitrogen concentration for each species by neighbor treatment. Error bars represent one standard error of the mean. Letters represent significant differences between treatments within each species ($\alpha<0.05$).

Nitrogen content was 57 ± 3.6mg plant^{-1} in *C. maculosa* and 17 ± 1.9mg and 42 ± 2.6mg plant^{-1} for *F. idahoensis* and *P. spicata* respectively. For all species, N content was 43, 37, and 31% lower under AM conditions for *C. maculosa*, *F. idahoensis* and *P. spicata* respectively, consistent with the lower biomass of AM plants (Figure 1a). The barrier treatment affected N content for *C. maculosa* and *F. idahoensis* (Table 7). Nitrogen content was higher in *C. maculosa* without a barrier, and the effect was greater under non-AM conditions (Figure 9). For *F. idahoensis* the effect of barrier was influenced by neighbor treatment as seen for total biomass (Figure 2). Barrier and AM treatments interacted to affect N content in *P. spicata* (Table 7). When grown with a barrier, *P. spicata* tended to have lower N content under AM conditions and higher N content under non-AM conditions compared with the no barrier treatment (Figure 9).

Table 7. Results of ANOVA for N content (g plant $^{-1}$). *C. maculosa* and *F. idahoensis* were log transformed, and *P. spicata* was square root transformed.

	C. maculosa			*F. idahoensis*		*P. spicata*	
	Residual df: 33			27		34	
Factor	df	F	p	F	p	F	p
AM	1	50.60	0.000	14.49	0.001	25.82	0.000
Neighbor	3	6.91	0.001	15.35	0.000	17.08	0.000
Barrier	1	10.43	0.003	15.49	0.001	0.07	0.791
AM X Neighbor	3	0.97	0.418	1.46	0.248	1.66	0.194
AM X Barrier	1	2.36	0.134	1.88	0.182	6.08	0.019
Neighbor X Barrier	3	0.41	0.749	10.05	0.000	2.65	0.064
AM X Barrier X Neighbor	3	0.21	0.892	0.92	0.444	0.11	0.954

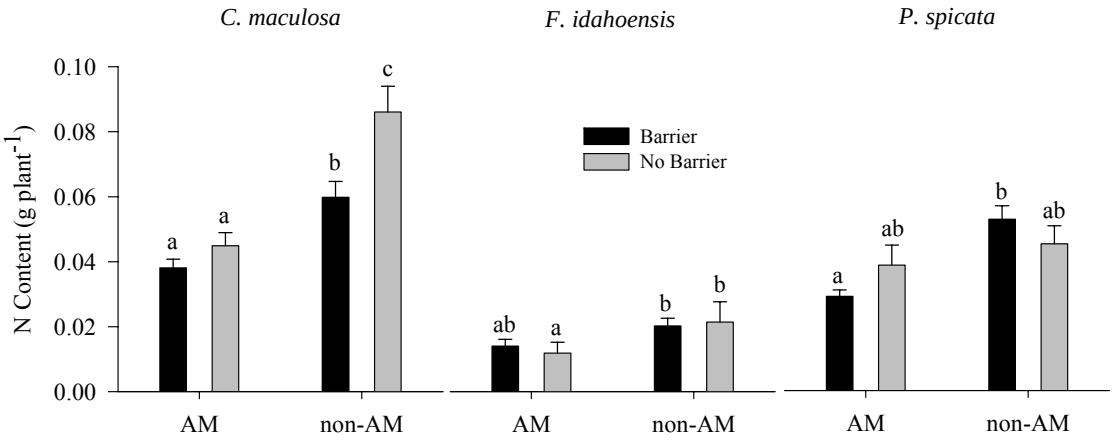

Figure 9. N content (g plant^{-1}) of each species by AM and barrier treatments. Error bars represent one standard error of the mean. Letters represent significant differences between treatments within each species ($\alpha<0.05$).

Plant Phosphorous

Phosphorous concentrations were 0.22 ± 0.01%, 0.23± 0.01% and 0.16 ± 0.004% for *C. maculosa, F. idahoensis, and P. spicata* respectively. The AM treatment affected P concentration only for *C. maculosa* (Table 8).

Table 8. Results of ANOVA for tissue percent P. No transformations were performed. Data were not available for *F. idahoensis* three-way interaction due to small plant size and insufficient plant material for P analysis for the AM, no barrier, and *C. maculosa* neighbor treatment.

	C. maculosa			*F. idahoensis*		*P. spicata*	
	Residual df:	33		30		34	
Factor	df	F	p	F	p	F	p
AM	1	90.97	0.000	1.38	0.250	0.04	0.837
Neighbor	3	0.09	0.967	0.02	0.894	1.63	0.200
Barrier	1	0.28	0.599	2.39	0.089	1.33	0.256
AM X Neighbor	3	1.05	0.381	0.20	0.656	2.80	0.055
AM X Barrier	1	0.20	0.659	2.11	0.120	2.28	0.140
Neighbor X Barrier	3	0.03	0.993	1.86	0.158	0.35	0.792
AM X Neighbor X Barrier	3	1.13	0.352	NA	NA	2.79	0.056

Plants had higher P concentration in the AM treatment (Figure 10). Neighbor and barrier main effects did not influence P concentration of any species (Table 8). A marginal three-way interaction affected *P. spicata* plant P concentration (Table 8). Phosphorous concentration was higher when paired with a *C. maculosa* neighbor than with a *P. spicata* neighbor under AM conditions, and the effect was most pronounced without a barrier present (Figure 11). No interactions between factors influenced P concentrations in *C. maculosa* or *F. idahoensis.*

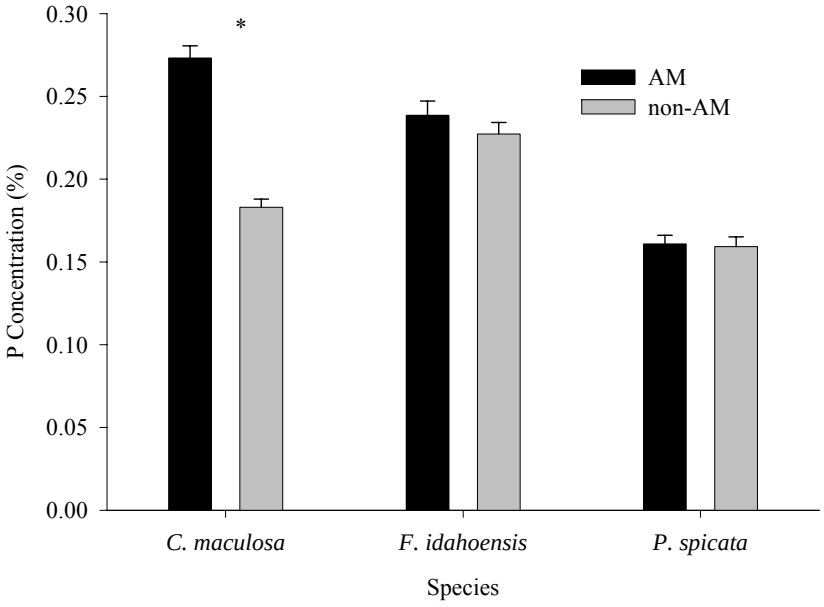

Figure 10. Percent P by AM treatment. Error bars represent one standard error of the mean. (*) Indicates significant difference between means of AM vs. non-AM treatments ($\alpha<0.05$).

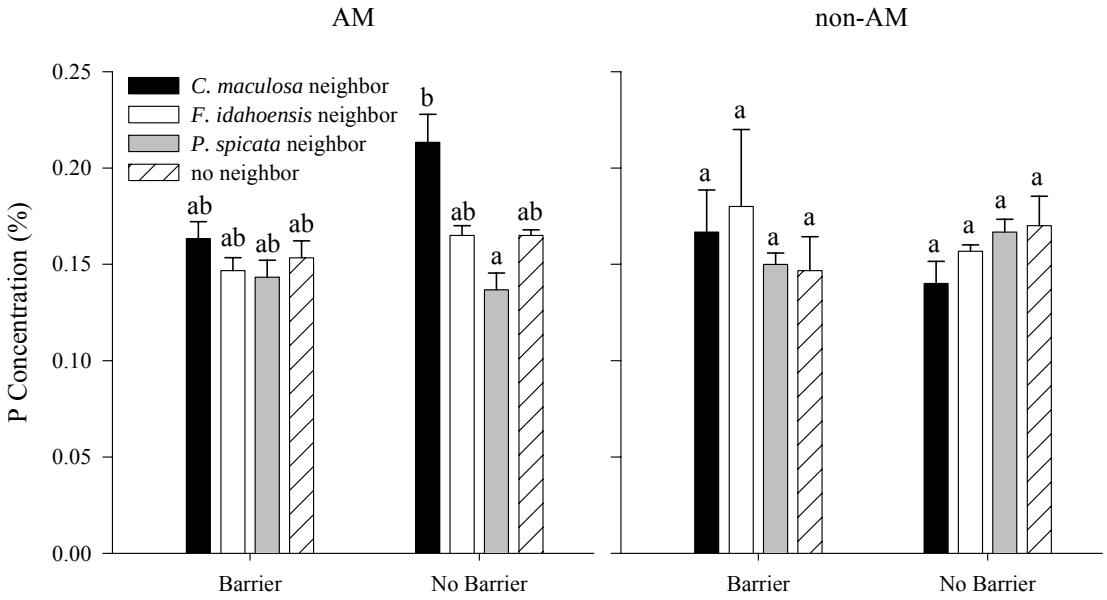

Figure 11. *Pseudoroegneria spicata* percent P by AM, barrier and neighbor interactions. Error bars represent one standard error of the mean. Letters represent significant differences between treatments within each species ($\alpha<0.05$).

Phosphorous content was 13 ± 0.68mg plant^{-1} in *C. maculosa* and 2.8 ± 0.30mg and 7.5 ± 0.44mg plant^{-1} in *F. idahoensis* and *P. spicata* plants, respectively. All species' P content was affected AM and neighbor factors, in a way that parallels each species biomass response (Table 9, Figure 1a & b). Phosphorous content was lower with the AM treatment and *C. maculosa* or *P. spicata* neighbors. Phosphorous content for *C. maculosa* and *P. spicata* was affected by an interaction of barrier and AM factors (Table 9). For *C. maculosa*, P content was higher without a barrier under non-AM conditions and the interaction for *P. spicata* was similar to that for N content (Figure 9). Neighbor and barrier treatments interacted similarly to affect P content as they affected total biomass (Figure 2).

Table 9. Results of ANOVA for P content (g plant^{-1}). *Centaurea maculosa* and *F. idahoensis* were log transformed and *P. spicata* was not transformed. Data were not available for *F. idahoensis* three-way interaction due to small plant size and insufficient plant material for analysis of the treatment of AM, no barrier, and *C. maculosa* neighbor.

	C. maculosa			*F. idahoensis*		*P. spicata*	
	Residual df:	33		30		34	
Factor	df	F	p	F	p	F	p
AM	1	10.89	0.002	12.90	0.001	35.07	0.000
Neighbor	3	9.08	0.000	4.32	0.046	11.16	0.000
Barrier	1	3.83	0.059	12.19	0.000	1.23	0.275
AM X Neighbor	3	0.64	0.593	0.04	0.852	0.06	0.982
AM X Barrier	1	9.28	0.005	1.65	0.199	6.90	0.013
Neighbor X Barrier	3	0.48	0.701	5.89	0.003	1.72	0.181
AM X Barrier X Neighbor	3	0.75	0.528	NA	NA	1.51	0.230

Discussion

Arbuscular mycorrhizal fungi improve plant growth and nutrition for most species of grassland plants (Smith & Read 1997). In this study, I found that AM fungi were not beneficial to plant growth, as plants in the AM treatment had significantly less biomass than non-AM plants. Negative effects of AM fungi on plant growth can occur if the cost of carbon given to the fungal symbiont is greater than the benefit of acquisition of nutrients (Koide & Elliott 1989). It is difficult to interpret, however, how much AM caused growth suppression for the grass species in this study, due to low levels of colonization (3% and 7% colonization).

The non-AM treatment was produced with pasteurized soil medium to kill AM propagules, with a microbial wash of smaller, non-AM fungi and bacteria added. The AM treatment soil may have had more pathogenic fungi than the non-AM treatment soil, resulting in depressed growth in the AM treatment. Another possibility is that the steam

sterilization increased nitrogen availability in the non-AM pots, which in turn led to greater biomass for these plants (Koide & Li 1989), a difference that can be especially important in very low nitrogen conditions such as ours (Thompson 1990).

I predicted that for plants grown singly, biomass would be lower in pots with a barrier, with the caveat that AM plants would not be affected by barrier because they have access through ERH to nutrients from the opposite side of the pot. In general the barrier did not limit plant growth for plants grown singly. *Centaurea maculosa* and *F. idahoensis* plants grown alone were the same size with and without a barrier present. *Festuca idahoensis* plants were so small, however, (0.9g) that they were probably not limited by resources in half the pot volume.

For *C. maculosa,* the barrier likely did not affect biomass because dissolved nutrients such as nitrogen compounds could have crossed the barrier through mass flow and diffusion. Nitrogen content data supports this interpretation since it usually did not differ between plants with or without a barrier, for both AM and non-AM plants (Figure 12).

The lack of response of *C. maculosa* biomass to the barrier could also reflect that it had significant amounts of ERH. Arbuscular mycorrhizal plants could have accessed immobile nutrients across the barrier, however P content was not as high in AM plants as it was in non-AM plants, regardless of barrier treatment. Either AM hyphae were not involved in access of nutrients across the barrier, or another effect of the AM treatment, such as pathogens, masked the effect of AM hyphae. Alternatively, some P could be accessed across the barrier through roots. In studies on AM hyphal transport of P,

experiments using similar mesh compartments have found that roots can deplete soil P up to one centimeter into the hyphal compartment (Jakobsen *et al.* 1992) and the barrier utilized in this study had a large surface area.

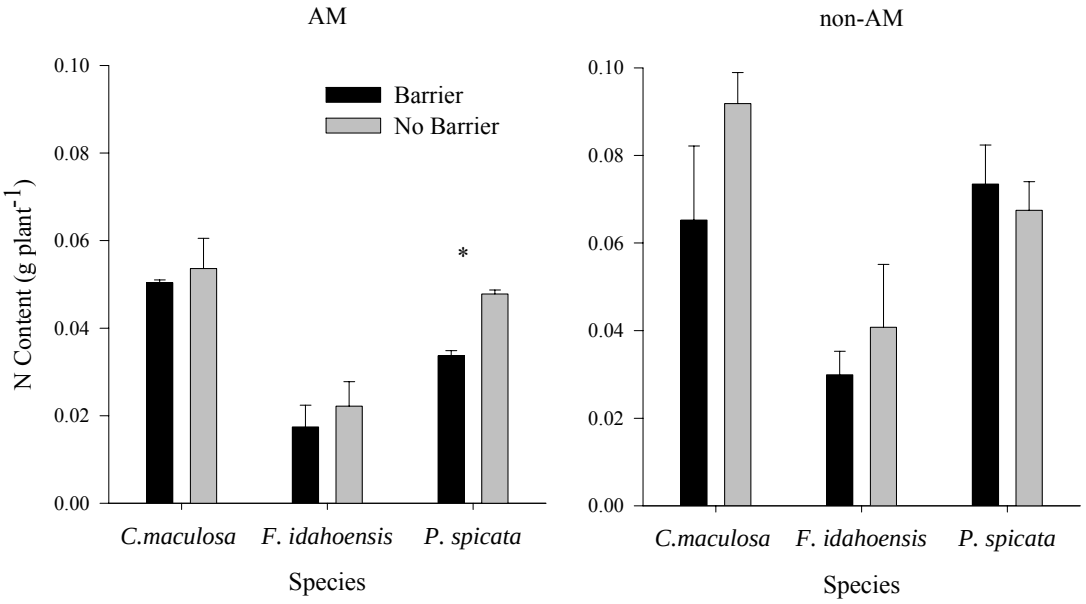

Figure 12. Nitrogen content of each species grown alone with and without a barrier for AM and non-AM plants. Error bars represent one standard error of the mean. (*) Indicates significant difference between means of AM vs. non-AM treatments ($\alpha<0.05$).

The barrier did not affect *P. spicata* biomass grown alone under non-AM conditions as was seen for *C. maculosa* and *F. idahoensis*, but with AM, both total biomass and shoot biomass were reduced in pots with the barrier. This result was also observed by Moora and Zobel (1998). Root mass ratio generally decreases with increased nutrient availability (Fitter 1997), and *P. spicata* had access to more nutrients with more space when grown without the barrier, as reflected in higher N content (Figure 12). Under non-AM conditions, where biomass was not affected by barrier, *P. spicata* plants were much larger than AM plants and potentially the increased transpiration of a

larger plant allowed for increased mass flow of dissolved nutrients across the barrier. Again, nitrogen content data indicates that *P. spicata* plants had as much nitrogen uptake with the barrier as without the barrier (Figure 12) indicating that plants were probably able to access nitrogen through mass flow across the barrier.

I hypothesized that the barrier treatment would allow belowground competitive interactions in mycorrhizal plants via ERH, but competition (reduction in biomass when grown with a neighbor) occurred with the barrier present for all species with and without AM fungi. The effect of varying neighbor species was much stronger. For *C. maculosa* focal plants, *C. maculosa* and *P. spicata* neighbors reduced *C. maculosa* biomass as compared with growing alone. A *F. idahoensis* neighbor also reduced *C. maculosa* biomass, but only under non-AM conditions. These effects could be due to shoot competition, or an inability to access dissolved nutrients, as occurred with plants growing alone, because they are used by the neighbor plant.

Festuca idahoensis biomass was lower with a *P. spicata* neighbor across the barrier, for similar reasons discussed for *C. maculosa*. With a *C. maculosa* neighbor, biomass was reduced only under AM conditions, which could be the result of additional nutrient depletion by *C. maculosa* AM hyphae. The trend was similar for non-AM plants, however, and with large variances in *F. idahoensis* biomass statistical differences were difficult to detect. Conspecifics did not affect *F. idahoensis* biomass, likely because the two *F. idahoensis* plants were not large enough that resources became limiting. All neighbors reduced *P. spicata* biomass with a barrier present, regardless of AM treatment.

This is probably because of competition for nutrients as seen for *C. maculosa*, or through AM hyphae when grown with a *C. maculosa* neighbor.

A greater reduction in the biomass of grasses grown with a neighbor was observed when the barrier was removed and root contact could occur. When paired with *C. maculosa*, the grass species were significantly smaller without the barrier present, indicating that either root contact is important to *C. maculosa*'s competitive effect on the native grasses, or that *C. maculosa* simply grew faster and was able to occupy space more quickly than the grasses (McConnaughay & Bazzaz 1991). Additionally, *C. maculosa* allocated more biomass to its root system than the grasses, which could explain why *C. maculosa* was a more successful competitor with direct root competition, as plants with higher RMR are often more competitive (Aerts *et al.* 1991).

Allelopathy could be involved in the more negative effect of *C. maculosa* on native grasses without the barrier (Bais *et al.* 2003), however, a similar reduction in *F. idahoensis* biomass was observed with a *P. spicata* neighbor under AM conditions. While allelopathy could be occurring with a *C. maculosa* neighbor, there is no documentation of the allelopathic chemical (-)-catechin production in *P. spicata*. Also, water soluble allelopathic chemicals should be able to cross the mesh barrier, but *C. maculosa* had a large effect on grass biomass only without the barrier. In contrast to the effect on grass species, the effect of a neighbor on *C. maculosa* biomass was independent of barrier presence. Biomass was lower with a neighbor with and without a barrier present.

Total pot biomass (summing the responses of both plants) was greater for *C. maculosa* and *P. spicata* grown together when a barrier was present, while the total pot biomass of either two *C. maculosa* plants or two *P. spicata* plants was not affected by barrier presence. This is counter to the findings of Falik (2003) that plants produce more biomass in the presence of a competitor than in competition with itself. These results suggest that the potential for root contact increases competitive effects between these two species, a result consistent with either allelopathic effects or plant detection of a non-self plant which results in increased interference competition.

This experiment has documented numerous potential traits that contribute to *C. maculosa*'s competitive ability, including increased nutrient concentration with AM fungi present, increased AM colonization and high ERH density. *Centaurea maculosa* was the only plant to have higher concentrations of P with AM fungi. While this could be partly due to higher nutrient concentrations in smaller (AM) plants, the grass species were also smaller under AM conditions, but did not have higher nutrient concentrations. With higher AM colonization levels than the other two species (18% vs. 3 and 7% colonization), *C. maculosa* may have reached a threshold level of colonization, at which the benefits of colonization outweighed the costs, at least in terms of nutrient acquisition (Gange & Ayres 1999)

Another explanation for increased nutrient concentration in *C. maculosa* in the AM treatment is its high ERH density. It was the only species to have a significant density of ERH, aside from *F. idahoensis* when it was grown with a *C. maculosa* neighbor, and also the only species to have greater P concentration in the AM treatment.

Without significant ERH density, it is not surprising that AM fungi did not have a positive effect on grasses. Phosphorous uptake has not successfully been correlated with AM colonization levels, but there is evidence that ERH density is more important in predicting P uptake by AM fungi than AM colonization (Jakobsen *et al.* 2001), so high ERH density can be extremely advantageous to plants.

The barrier influence on nutrient uptake differed for N and P in *C. maculosa* plants. Concentration of P in *C. maculosa* was higher in the AM treatment than the non-AM treatment for both barrier treatments, whereas for N concentration, it was only higher with AM fungi without the barrier present. This suggests that although AM hyphae access N (Frey & Schüepp 1993), hyphae did not significantly aid plants in accessing N unavailable to roots, while P acquisition through the barrier via ERH was more efficient.

Our experimental conditions were such that N was likely more limiting to plant growth than P because N:P ratios of plant tissue ranged from 4.5 in *C. maculosa* to 6.5 in *F. idahoensis* (data not shown), which is well below the optimal level of 16 (Koerselman & Meuleman 1996). If N is the most limiting nutrient, then those plants with the greatest N content are the most competitive plants, and additional access to P did not greatly aid *C. maculosa* in terms of biomass. In our experiment, non-AM *C. maculosa* plants grown without a barrier had the greatest N content (Figure 12). Although I predicted that AM colonization would aid the most competitive plant, it appears that an extensive root system was more important to accessing nutrients. *Centaurea maculosa* increased its root length and therefore absorptive surface area in the non-AM treatment more than the native grasses were able to (Figure 13).

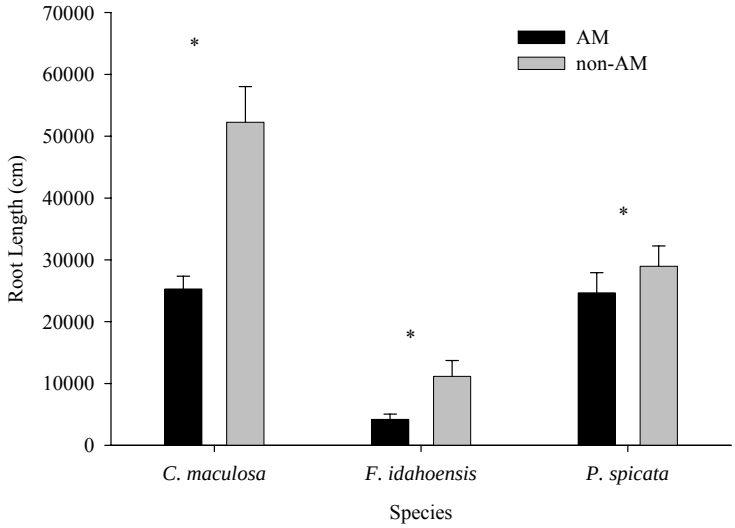

Figure 13. Root length (cm) of each species by AM treatment. Error bars represent one standard error of the mean. (*) Indicates significant difference between means of AM vs. non-AM treatments ($\alpha<0.05$).

The high AM colonization in *C. maculosa* plants also affected colonization in neighboring plants. *Festuca idahoensis* had higher colonization with a *C. maculosa* neighbor than with any other neighbor species or growing alone, and *P. spicata* had the same trend, although colonization with a *C. maculosa* neighbor was only significantly greater with a *F. idahoensis* neighbor. Grass biomass production was greatly reduced in this treatment, indicating that increased colonization could have been a greater carbon drain on these plants, reducing biomass. Interestingly, *C. maculosa* had lower colonization without the barrier treatment. Although not statistically significant, the grasses also tended to have higher colonization with a *C. maculosa* neighbor without the barrier present, which may explain why *P. spicata* P concentration was greater with a *C. maculosa* neighbor under AM conditions without a barrier present.

One explanation for *C. maculosa's* success could be the release from soil borne pathogens in new locations (Callaway *et al.* 2004). There is evidence that non-native plants do not accumulate pathogenic fungi as quickly as native plants, giving them an advantage when they colonize new areas (Goldberg 1990; Klironomos 2002). In our study, however, both natives and the non-native had reduced biomass in the presence of AM fungi (and pathogens), indicating that if pathogens were responsible for that effect, it was not expressed in native plants only. All three species were present on the site where field soil was obtained, so potentially *C. maculosa* also had a negative soil feedback (Bever *et al.* 1997).

In this study I found that AM benefits *C. maculosa* in terms of increased nutrient acquisition. Root contributions to competitive ability were also evident by reduced biomass of neighboring grasses without a barrier present. *Centaurea maculosa* also had greater AM fungal colonization, and ERH density than the native grasses. This is likely why *C. maculosa* had increased nutrient concentration under AM conditions, and could lead to increased size, seed production (Gange & Ayres 1999; Shumway & Koide 1994) and success in invading undisturbed semi-arid rangelands.

3. ARBUSCULAR MYCORRHIZAE AND NEIGHBOR EFFECTS ON *C. MACULOSA* AND NATIVE GRASS RESPONSE TO HERBIVORY

Introduction

The exotic plant species *Centaurea maculosa* has invaded over four million hectares of native rangelands and grasslands in the western United States. Its establishment poses serious ecological and economic impacts. Management of *C. maculosa* includes mechanical or chemical removal, insect biocontrol agents and grazing. Successful implementation of management tools can be strengthened by knowledge of the biology of *C. maculosa,* including factors influencing its response to herbivory.

The use of grazing to control *C. maculosa* requires that neighbor species have greater competitive ability after defoliation of *C. maculosa* (Olson 1999). If *C. maculosa* can replace lost tissue faster than native grasses, or if competitive outcomes between the species are not altered with herbivory, grazing will not reduce *C. maculosa* abundance. *Centaurea maculosa* increases biomass production after herbivory (Callaway *et al.* 1999) and release additional allelopathic chemicals (Thelen *et al.* 2005). These studies focused on root herbivory, however, and a recent study on shoot herbivory has found no evidence of greater allelopathy following shoot herbivory (Newingham & Callaway)).

Arbuscular mycorrhizal fungi readily colonize native plants and the invasive *C. maculosa* in semi-arid grasslands (Marler *et al.* 1999b), and AM fungi influences competition between *C. maculosa* and *F. idahoensis* (Marler *et al.* 1999a), however, there was no evidence of AM influence on competition in Chapter 2. Plant response to herbivory has also been linked to AM fungi (Gehring & Whitham 1994), so both factors

are important considerations when evaluating weed management with insect biocontrols or grazing.

Marler *et al.* (1999a) showed that while AM fungi did not directly influence the biomass of either *C. maculosa* or *F. idahoensis* grown alone, however when these species were grown together with AM fungi, *F. idahoensis* biomass was reduced, or *C. maculosa* biomass increased, relative to plants in the non-AM treatment. Mechanisms for how AM fungi could influence competition include accessing carbon or nutrients from neighboring plants through linkages of extraradical hyphae (Grime *et al.* 1987; Simard *et al.* 1997); differing effects of AM on host plants (Hartnett *et al.* 1993; Hartnett & Wilson 2002; Zabinski *et al.* 2002), and indirectly through effects on plant interactions with herbivores, pathogens or other soil microorganisms (Eom *et al.* 2001; Gehring & Whitham 1994; Hodge 2000).

Foliar feeding invertebrates and grazing ungulates in general have a negative effect on a plant's ability to grow and reproduce, which influences the competitive ability of susceptible species. Herbivory due to grazing causes loss of photosynthetic area and nutrients, which can lead to decreased root growth (Crider 1955) and reduced plant productivity and fitness (McNaughton 1983). Secondary metabolites are produced in some plants to discourage grazing, while other plants tolerate grazing through compensatory growth of lost foliage, by allocating stored carbon to regrowth. The ability to compensate for herbivory depends upon the timing and plant's growth stage at the time of defoliation (Turner *et al.* 1993), the severity of defoliation (Crawley 1997), and nutrient and water availability (Chapin & McNaughton 1989). Plant species differ in

their abilities to avoid or tolerate herbivory, which may influence the outcome of interspecific plant competition (Chapin & McNaughton 1989) and plant community structure.

Arbuscular mycorrhizal (AM) fungal associations are present in most (80%) terrestrial plant species (Smith & Read 1997) and are likely associated with compensatory response to herbivory (Gehring & Whitham 1994) because of the role of AM for acquiring nutrients necessary for plant growth. A plant's compensatory growth could be increased by AM fungi by increasing nutrient availability, or decreased by acting as a sink for carbon that could otherwise be allocated to compensatory growth. This influence on compensatory response could later alter the outcome of competition between native and weedy species.

The effects of herbivory on mycorrhizae, specifically AM colonization levels, range from negative, (Bethlenfalvay *et al.* 1985; Borowicz & Fitter 1990; Trent *et al.* 1988) to neutral, (Daft & El-Giahmi 1978; Del Vecchio *et al.* 1993; Wallace 1987; Walling & Zabinski 2005) to positive effects (Eom *et al.* 2001; Kula *et al.* 2005; Wallace 1981; Wallace 1987) The variation in response can be explained by the fact that the interaction varies with plant and fungal species, and environmental variables. Negative effects of herbivory on AM colonization levels are likely due to a decrease in photosynthate after defoliation, which reduces the carbon allocated to mycorrhizae (Bever & Dakessian 1984). Arbuscular mycorrhizal fungi are beneficial to a host plant only if the benefit it receives is greater than the carbon cost necessary for maintaining the

symbiont. It has been estimated that AM fungi utilize approximately 10-20 percent of a plant's total photosynthate (Jakobsen *et al.* 2002).

This experiment investigated the effects of AM fungi, neighboring species and herbivory (simulated by clipping) on plant fitness as measured by biomass response and nutrient status. The study will expand on previous work by Marler *et al.* (1999a), by including a clipping treatment, and adds to work by Walling and Zabinski (Walling & Zabinski 2005) by including a neighbor species. The objectives of this experiment were to determine: 1) whether AM influences biomass of *C. maculosa* and native grasses when grown together; 2) whether clipping alters the effect of AM fungi on host plant competitive ability; 3) whether AM fungi enhances compensatory growth in *C. maculosa* or the native grasses when grown with a neighbor; and 4) how herbivory simulated by clipping affects AM colonization, ERH density and effectiveness in increasing plant nutrient uptake and subsequent compensatory growth.

Methods

Experimental Setup

This experiment was a randomized complete factorial design, including three species combinations, three clipping treatments, two AM treatments, and nine replicates of all treatments. *Festuca idahoensis var. winchester* (hereafter *F. idahoensis*), *P. spicata* and *C. maculosa* were all grown with a *C. maculosa* neighbor.

Festuca idahoensis, P. spicata, and *C. maculosa* were seeded directly into 7500 ml pots and thinned to one seedling after all plants were established. *Festuca idahoensis* was seeded 22 days before seeding *P. spicata* or *C. maculosa* because of its longer

germination and establishment time. Seedlings were transplanted into pots where establishment did not occur.

The soil medium used was a 4:1 sand:soil mix, with 30 grit silica sand and pasteurized loam topsoil. Mycorrhizal inoculum was added as unpasteurized field soil in a 1.5 cm layer 4 cm below the surface. The field soil inoculum also included roots of all three species in relatively equal proportions chopped small enough to pass through a 0.6cm screen. For non-AM treatment plants, the field soil was aerated steam pasteurized at 80 °C for 90 minutes before the layer was added to non-AM pots. To reintroduce non-AM microbes, 400 ml of field soil was mixed with 3000 ml of DI water and vacuum-filtered to pass through a number 1 Whatman filter (11µm) three times. Thirty ml of microbial wash was added to each non-AM treatment pot.

Plants were clipped 86 days after seeding *C. maculosa* and *P. spicata*. One plant per pot was clipped, or no plants were clipped to remove 75% of above ground biomass. Grass biomass was removed using a utilization scale to estimate the clipped height of plants equal to 25% of the biomass. (Bureau of Land Management 1996). *Centaurea maculosa* biomass was removed after visually estimating 75% of the plant's aboveground biomass.

Plants were grown for 17 weeks (33 days after clipping) under greenhouse conditions maintained at a 21 ºC daytime and 16 ºC nighttime temperature with supplemental lighting (GE Multi-Vapor MVR1000/C/U) as necessary for a 16 hour day length. Field soil was collected adjacent to the Red Bluff Research Ranch near Norris, Montana in an *F. idahoensis, P. spicata* dominated grassland with *C. maculosa* present,

and sieved through a 0.6cm screen. The soil mixture had initial NO_3^- and P levels of 9.7mg/kg and 4.3mg/kg respectively. I added 100ml of 1/2 strength Hoagland's solution to each pot four times during the experiment as plants showed signs of deficiency.

Plant Analysis

Root and shoot tissue was separated, cleaned and dried to a constant weight, and biomass was measured. Three plants of each species were randomly chosen from each treatment for tissue nutrient analysis. Root and shoot tissue was combined and ground to pass through a 0.25μm screen (UDY mill Model #3010-030; Fort Collins, CO, USA). Kjeldahl nitrogen and nitric acid/hydrogen peroxide digestion were performed, then analyzed for total N and P using Inductively Coupled Plasma Spectrometry (MDS Harris, Lincoln, NE, USA).

Mycorrhizal Analysis

To determine colonization levels in mycorrhizal and non-mycorrhizal treatments, fine root samples of five randomly selected replicates of plants from the AM treatment and two replicates of non-AM treatment plants were cleared for 48 hours in 2.5% KOH, rinsed with distilled water, acidified for 12 hours with 3% HCL, and stained for 12 hours with 0.05% Trypan blue solution (Phillips & Hayman 1970). Mycorrhizal structures including internal hyphae, vesicles and arbuscules were quantified using the magnified line intersect method (McGonigle *et al.* 1990).

Extraradical hyphae were quantified from two 2.5 cm diameter soil cores extracted from each pot. Soil cores were located 3 cm from the base of each plant.

Extraradical hyphae was extracted from these samples using a modified method of Miller *et al.* (1995). Soil cores were homogenized, and a 6g sample was mixed with 15 ml of 3.75% sodium hexametaphosphate and 100ml water, stirred for 5 minutes and allowed to sit for 30 minutes to dissociate soil particles. A sample of each soil was dried to obtain dry weight equivalents. A 10ml aliquot was diluted with 100ml water, and 20ml was removed and filtered through a 20μm mesh filter. The filter was placed in a centrifuge tube and stained with 5 ml 0.5% Trypan blue for five minutes. The contents were filtered onto a 0.45μm membrane filter. AM hyphae were distinguished from non-AM hyphae under 200x magnification by characteristics including diameter, the presence of angular projections and a general lack of septae (Mosse 1959; Nicolson 1959; Sylvia 1992). The length of hyphae on each filter was estimated using a grid line intersect method (Tennant 1975) to calculate length of hyphae g^{-1} soil.

Statistical Analysis

Variables including root, shoot and total biomass, root mass ratio, AM percent colonization, ERH density, and N and P concentration and content were analyzed with ANOVA using R 2.0.1 (R Development Core Team 2004). Three-way ANOVAs for the factors of AM (+/-), clipping (focal plant clipped, neighbor plant clipped, or no clipping), and neighbor species (*C. maculosa, F. idahoensis, P. spicata*) and interactions were analyzed for each variable individually for *C. maculosa*. Two-way ANOVAs were performed for *F. idahoensis* and *P. spicata* for the AM and clipping factors. Power transformations were identified using the Box-Cox procedure and equality of variance

was verified with the modified Levene's test. Tukey's multiple comparison procedure was used to separate means (α=0.05).

Results

Biomass

Total biomass for each species grown with a *C. maculosa* neighbor was 4.72g ± 0.41, 7.54g ± 0.46 and 3.34g ± 0.26 for *C. maculosa*, *F. idahoensis* and *P. spicata*, respectively. The AM treatment did not affect biomass for any species (Table 10), but there was a trend of higher biomass with the AM treatment for *F. idahoensis* and *P. spicata* (Figure 14a).

Table 10. Results of ANOVA for total biomass (g). All plants were grown with a *C. maculosa* neighbor. Data were not transformed for *C. maculosa* or *F. idahoensis* and were square root transformed for *P. spicata*.

		C. maculosa		*F. idahoensis*		*P. spicata*	
Factor	Df	F	p	F	p	F	p
AM	1	1.46	0.232	2.85	0.098	3.27	0.077
Clipping	2	10.83	0.000	13.04	0.000	3.40	0.042
AM X Clipping	2	0.41	0.668	4.01	0.025	0.34	0.713
Residual Df		48		48		47	

The clipping treatment affected biomass of all three species (Table 10). For both *C. maculosa* and *F. idahoensis* plants, biomass was greatest for unclipped plants and plants with clipped neighbors, and smallest when the focal plant was clipped (Figure 15). For *P. spicata*, plants were smallest when clipped and largest when the neighbor plant was clipped. There was no difference in size between clipped and unclipped *P. spicata* plants, or unclipped plants and plants with clipped neighbors (Figure 15).

An interaction of clipping and AM factors affected total biomass for *F. idahoensis* (Table 10). Biomass tended to be greater when the *C. maculosa* neighbor was clipped than with no clipping, only with AM fungi present. In the non-AM treatment, *F. idahoensis* biomass tended to be lower when the neighbor was clipped (Figure 16). Clipped plants were smaller than unclipped plants only in the non-AM treatment. The effect of the clipping treatment did not vary by the AM treatment for any other species.

Centaurea maculosa was the only species grown with *F. idahoensis* and *P. spicata* neighbors, and neighbor species did influence *C. maculosa* biomass (Table 11). Biomass was greatest with a *P. spicata* neighbor and smallest with both *C. maculosa* and *F. idahoensis* neighbors (Figure 14b). Clipping and AM treatments interacted slightly to affect *C. maculosa* biomass (Table 11). When grown with *C. maculosa* neighbors, the biomass of clipped plants was smaller than unclipped plants in the AM treatment. When grown with a *F. idahoensis* neighbor, clipped plants had smaller biomass than unclipped plants in the non-AM treatment. Unclipped plants were larger than clipped plants for both AM treatments when grown with a *P. spicata* neighbor.

Table 11. Results of ANOVA for *C. maculosa* total biomass (g) with all neighbors. Data were quarter root transformed.

Factor	Df	F	p
AM	1	0.80	0.371
Neighbor	2	22.75	0.000
Clipping	2	34.46	0.000
AM X Neighbor	2	0.64	0.530
AM X Clipping	2	0.84	0.432
Neighbor X Clipping	4	1.07	0.372
AM X Neighbor X Clipping	4	2.02	0.094
Residuals	145		

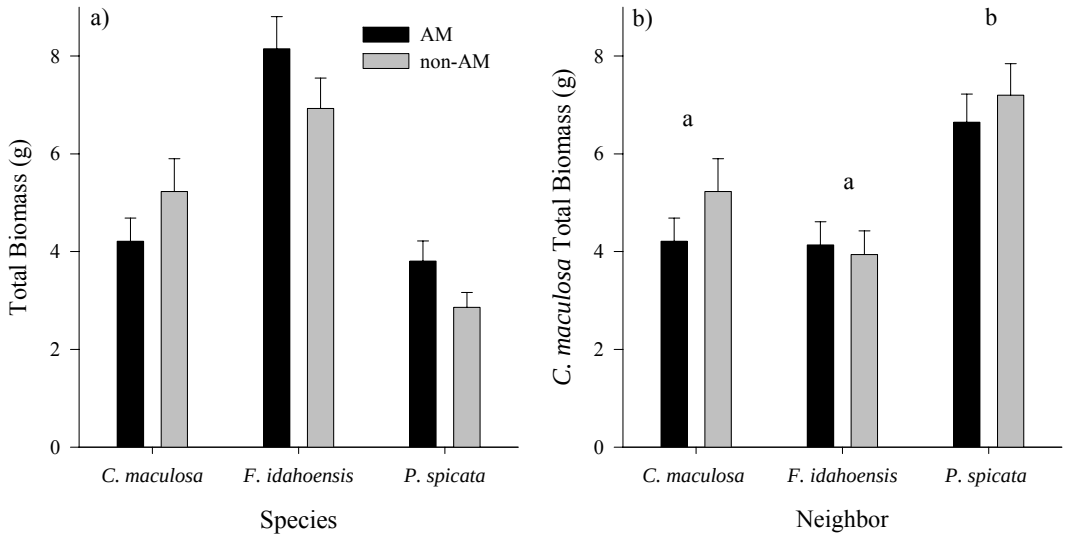

Figure 14. a) Total biomass (g) for each species by AM treatment. All species were grown with a *C. maculosa* neighbor. Error bars represent one standard error of the mean. There were no significant differences within species b) Neighbor and AM treatment effects on *Centaurea maculosa* total biomass. Letters represent significant differences between treatments within each neighbor species ($\alpha<0.05$).

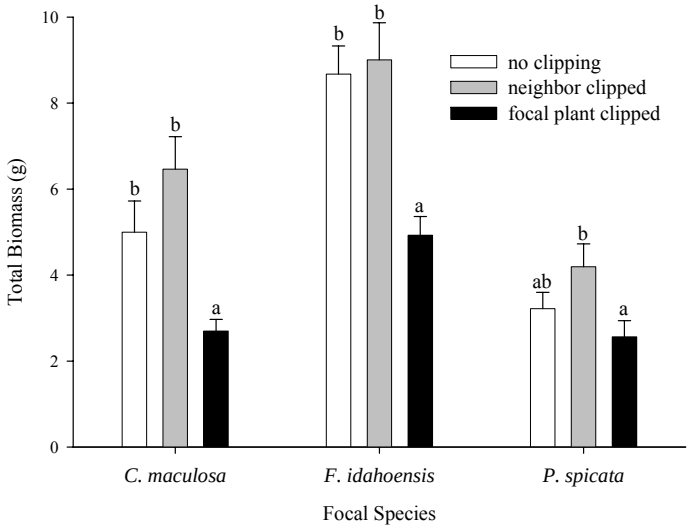

Figure 15. Clipping treatment effects on total biomass (g) for each species. All species were grown with a *C. maculosa* neighbor. Error bars represent one standard error of the mean. Letters represent significant differences between treatments within each neighbor species ($\alpha<0.05$).

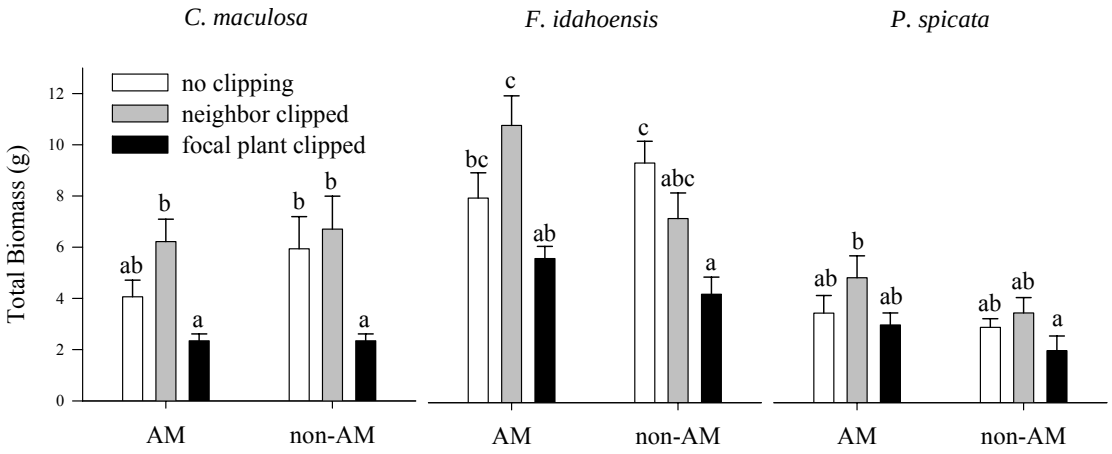

Figure 16. Clipping and AM treatment effects on total biomass (g) for each species growing with *C. maculosa*. Error bars represent one standard error of the mean. Letters represent significant differences between treatments within each neighbor species ($\alpha<0.05$).

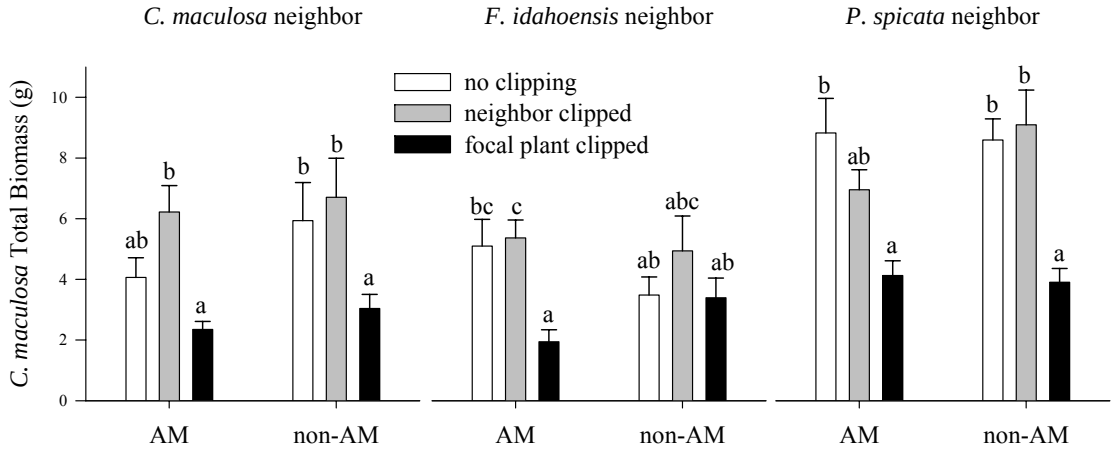

Figure 17. Clipping and neighbor treatment effects on *C. maculosa* total biomass (g) under AM and non-AM conditions. Error bars represent one standard error of the mean. Letters represent significant differences between treatments within each neighbor species ($\alpha<0.05$).

RMR

The root mass ratio (RMR) of each species was 0.51, 0.55 and 0.64 for *C. maculosa*, *F. idahoensis* and *P. spicata,* respectively. The AM treatment affected RMR of only *C. maculosa* (Table 12), with lower root mass ratio in the AM treatment (Figure 18). The clipping treatment did not affect RMR for any species grown with *C. maculosa* (Table 12). For *F. idahoensis*, RMR was affected by an interaction of AM and clipping factors (Table 12). Root mass ratio tended to be lower in the AM treatment for unclipped plants and higher in the AM treatment for plants with clipped neighbors and clipped plants (Figure 20).

Table 12. Results of ANOVA for RMR. All plants were grown with a *C. maculosa* neighbor. No transformations were performed.

		C. maculosa		*F. idahoensis*		*P. spicata*	
Factor	Df	F	p	F	p	F	p
AM	1	4.04	0.050	0.45	0.504	1.30	0.260
Clipping	2	1.92	0.158	0.75	0.480	0.01	0.991
AM X Clipping	2	1.62	0.208	6.43	0.003	1.97	0.151
Residual Df		48		48		47	

For *C. maculosa* plants grown with all neighbors, RMR was affected by AM, neighbor and clipping treatments (Table 13). Root mass ratio was highest for *C. maculosa* when grown with a *F. idahoensis* neighbor (Figure 18). The clipping treatment affected *C. maculosa* RMR (Table 13) with higher RMR in clipped plants (Figure 19). Although not statistically significant, the same trend occurred for the grass species grown with a *C. maculosa* neighbor (Figure 19).

Table 13. Results of ANOVA for *C. maculosa* RMR. Data were not transformed.

Factor	Df	F	p
AM	1	6.21	0.014
Neighbor	2	11.87	0.000
Clipping	2	4.52	0.012
AM X Neighbor	2	0.38	0.687
AM X Clipping	2	0.90	0.408
Neighbor X Clipping	4	0.41	0.801
AM X Neighbor X Clipping	4	0.57	0.682
Residuals	145		

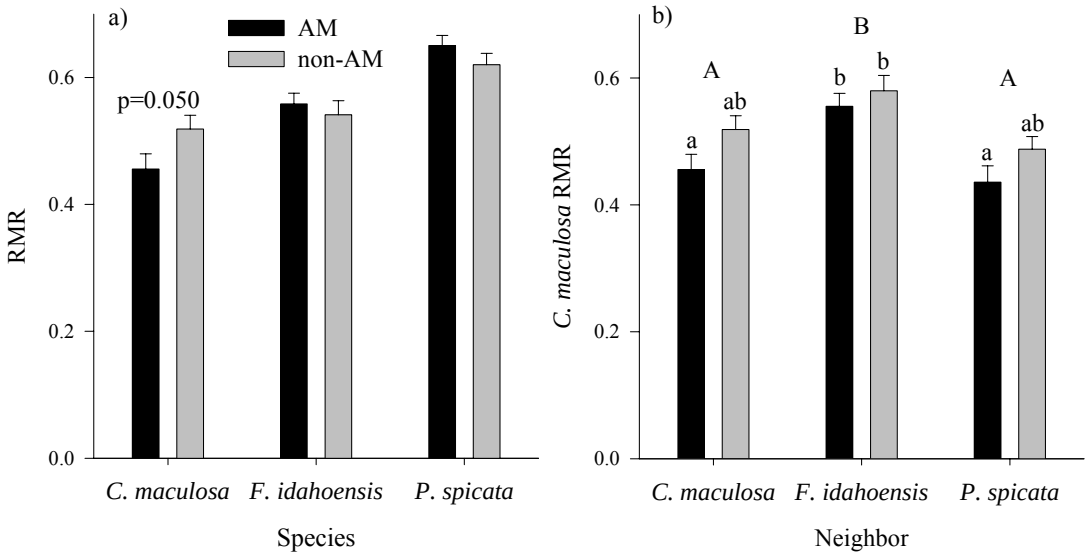

Figure 18. a) AM effect on RMR for each species grown with *C. maculosa* neighbors. *p*-value is for Tukey's multiple comparisons. b) Neighbor and AM effects on RMR of *C. maculosa*. Error bars represent one standard error of the mean. Lowercase letters represent significant differences between treatments within each neighbor species and capital letters represent significant differences between neighbors ($\alpha<0.05$).

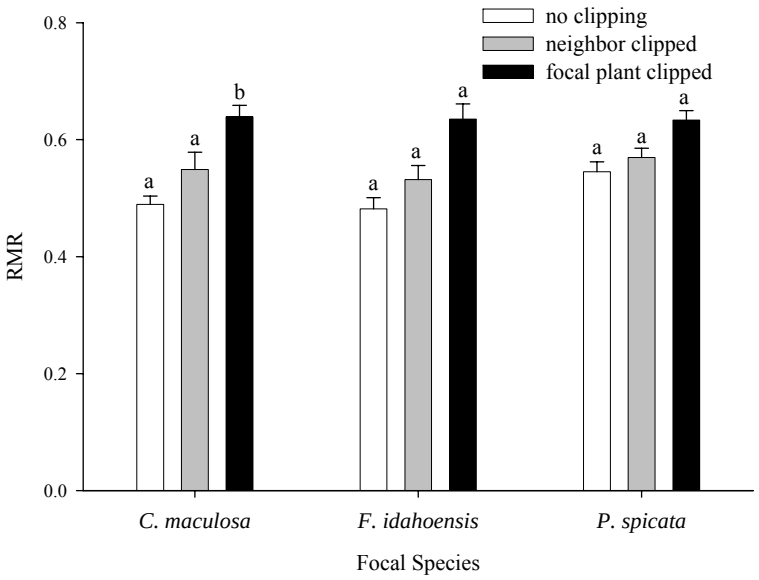

Figure 19. Clipping treatment effects on RMR for each species. *C. maculosa* was grown with all neighbors. Error bars represent one standard error of the mean. Letters represent significant differences between treatments within each neighbor species ($\alpha<0.05$).

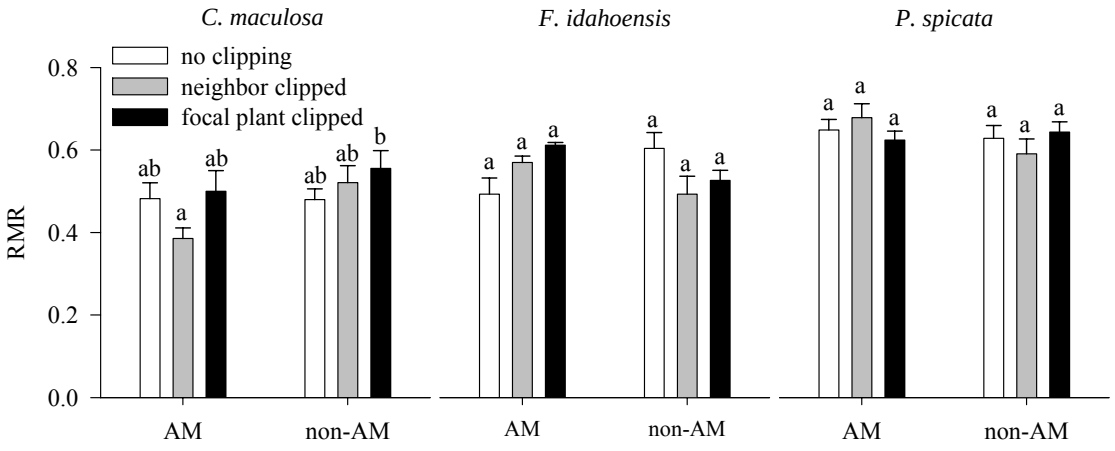

Figure 20. Clipping and AM effects on RMR of each species. Error bars represent one standard error of the mean. Letters represent significant differences between treatments within each neighbor species ($\alpha<0.05$).

AM Colonization

Colonization levels for each species were 18.0 ± 0.1%, 9.0 ± 3.1% and 6.5 ± 1.4% for *C. maculosa, F. idahoensis* and *P. spicata*, respectively. Clipping treatment did not affect colonization levels for *C. maculosa* or *P. spicata*, but colonization levels in *F. idahoensis* were influenced by clipping treatment (Table 14). Colonization was higher in unclipped *F. idahoensis* plants than in plants with clipped neighbors or in clipped plants (Figure 21a). Neither neighbor species, nor clipping treatment influenced AM colonization of *C. maculosa* (Table 15, Figure 21a & b).

Table 14. Results of ANOVA for AM Colonization (%) of *F. idahoensis* and *P. spicata* roots. Data for *F. idahoensis* was square root transformed and no transformations were performed for *P. spicata* data.

		C. maculosa		*F. idahoensis*		*P. spicata*	
Factor	Df	F	p	F	p	F	p
Clipping	2	0.89	0.438	5.68	0.016	0.67	0.531
Residual Df		11		14		12	

Table 15. Results of ANOVA for AM Colonization (%) of *C. maculosa* roots. Data were not transformed.

Factor	Df	F	p
Neighbor	2	0.42	0.658
Clipping	2	0.28	0.757
Neighbor X Clipping	4	0.42	0.795
Residuals	20		

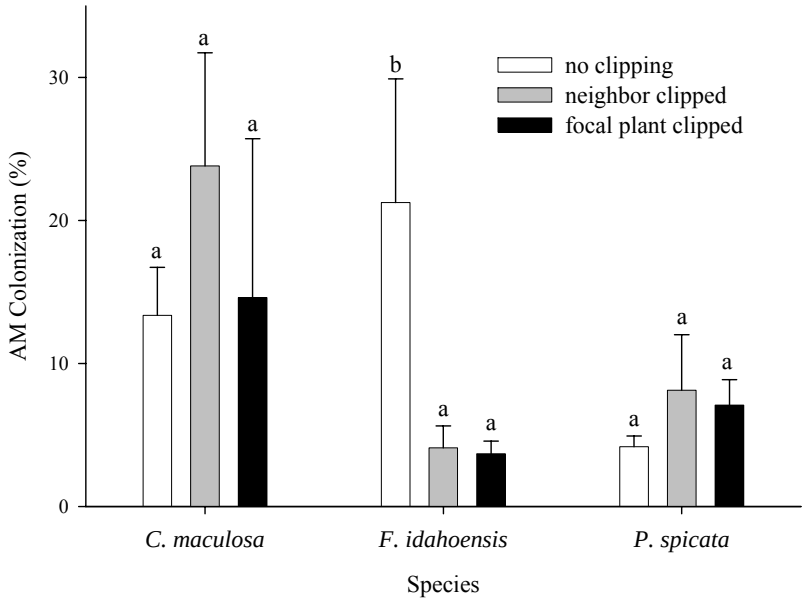

Figure 21. Clipping treatment effects on AM colonization of each species. Error bars represent one standard error of the mean. Letters represent significant differences (95% confidence) between treatments within each species. No significant differences between neighbors were present ($\alpha<0.05$).

Extraradical Hyphae

A background level of 2.14 ± 0.19 m hyphae g^{-1} soil was present in non-AM pots. For AM plants ERH density (reported here as total hyphal length density – background level) was 1.6 ± 0.2, 1.7 ± 0.3 and 1.6 ± 0.2 m g^{-1} for *C. maculosa*, *F. idahoensis* and *P. spicata* respectively. Samples were collected near the focal plant, but are likely a measure of focal and neighbor plant's hyphae. The clipping treatment did not affect ERH density for any species (Table 16, Figure 21b). No significant main effects or interactions were present for *C. maculosa* ERH density (Table 16), however there was a trend toward higher hyphal density with a *F. idahoensis* neighbor present. The ERH

density in *C. maculosa* pots was 1.4 ± 0.3, 2.0 ± 0.2 and 1.5 ± 0.4 for *C. maculosa*, *F. idahoensis* and *P. spicata* neighbors respectively.

Table 16. Results of ANOVA for ERH density of *F. idahoensis* and *P. spicata* roots. Data were not transformed.

		C. maculosa		*F. idahoensis*		*P. spicata*	
Factor	Df	F	p	F	p	F	p
Clipping	2	0.50	0.618	0.34	0.719	0.14	0.869
Residual Df		12		12		10	

Table 17. Results of ANOVA for ERH density of *C. maculosa* roots. Data were not transformed.

Factor	Df	F	p
Neighbor	2	2.61	0.088
Clipping	2	1.36	0.269
Neighbor X Clipping	4	1.53	0.214
Residuals	35		

Plant Nitrogen

Nitrogen concentrations (%) in plant tissues were 0.95 ± 0.04%, 0.77 ± 0.04% and 0.77 ± 0.04% for *C. maculosa*, *F. idahoensis*, and *P. spicata,* respectively. The AM treatment affected only *C. maculosa* N concentration (Table 18). Plants had lower N concentration in the AM treatment than the non-AM treatment (Figure 22). The clipping treatment also affected only *C. maculosa* N concentration (Table 18 & Table 19). Clipped plants had higher N concentration than unclipped plants (Figure 22).

For N content (g N plant^{-1}), the AM treatment did not have a significant effect on any species grown with a *C. maculosa* neighbor (Table 20). Nitrogen content of *F. idahoensis* was slightly influenced by clipping, whereas the other species' N content

did not differ between clipped and unclipped plants (Table 20). Unclipped plants had higher N content than clipped plants for *F. idahoensis* (Figure 23).

For *C. maculosa* plants grown with all neighbors, clipping and neighbor factors affected N content (Table 21). Plants had the highest N content with a *P. spicata* neighbor and lowest N content with a *F. idahoensis* neighbor, similar to the neighbor effect on total biomass. As for *F. idahoensis* plants, unclipped *C. maculosa* plants had higher N content than clipped plants (Figure 23).

Table 18. Results of ANOVA for N concentration. All plants were grown with a *C. maculosa* neighbor. Data were not transformed for any species

		C. maculosa		*F. idahoensis*		*P. spicata*	
Factor	Df	F	p	F	p	F	p
AM	1	5.46	0.048	0.65	0.444	0.12	0.737
Clipping	1	6.44	0.035	0.00	0.952	0.74	0.414
AM X Clipping	2	3.01	0.121	1.29	0.289	0.62	0.454
Residuals	8						

Table 19. Results of ANOVA for *C. maculosa* N concentration with all neighbors (g). Data were not transformed.

Factor	Df	F	p
AM	1	3.09	0.092
Neighbor	2	1.60	0.225
Clipping	1	9.57	0.005
AM X Neighbor	2	1.08	0.358
AM X Clipping	2	0.23	0.633
Neighbor X Clipping	4	0.46	0.638
AM X Neighbor X Clipping	4	1.82	0.184
Residuals	23		

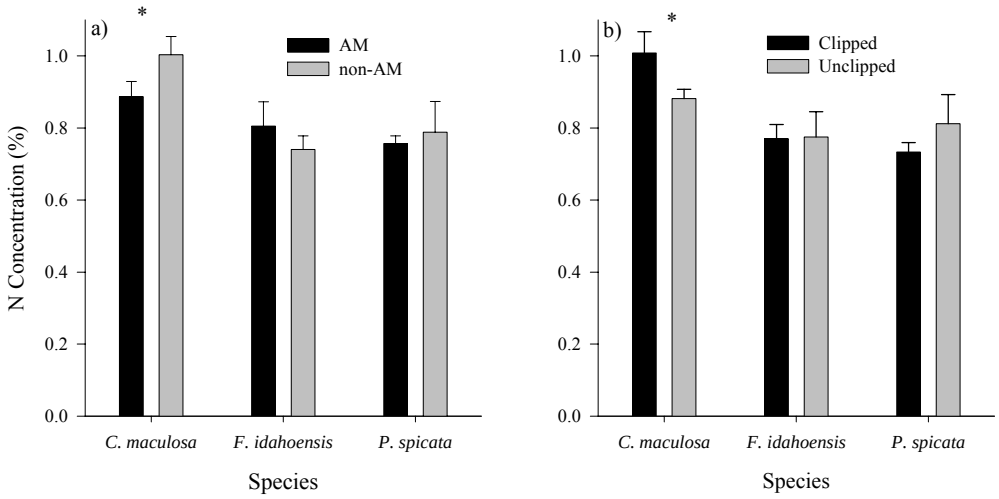

Figure 22. a) AM effect on N concentration of each species. Each species was grown with *C. maculosa*. b) Clipping effect on N concentration of each species. Each species was grown with *C. maculosa*. Error bars represent one standard error of the mean. (*) Indicates significant difference between means of AM vs. non-AM treatments ($\alpha<0.05$).

Table 20. Results of ANOVA for N content. All plants were grown with a *C. maculosa* neighbor. Data were not transformed for any species

		C. maculosa		*F. idahoensis*		*P. spicata*	
Factor	Df	F	p	F	p	F	p
AM	1	2.67	0.141	0.21	0.657	0.00	0.998
Clipping	1	1.09	0.326	5.23	0.051	1.06	0.337
AM X Clipping	2	0.45	0.523	2.43	0.158	0.03	0.859
Residuals	8						

Table 21. Results of ANOVA for *C. maculosa* N content with all neighbors (g). Data were not transformed.

Factor	Df	F	p
AM	1	0.21	0.652
Neighbor	2	6.63	0.005
Clipping	1	8.03	0.009
AM X Neighbor	2	2.80	0.081
AM X Clipping	2	0.00	0.983
Neighbor X Clipping	4	0.57	0.571
AM X Neighbor X Clipping	4	1.18	0.325
Residuals	24		

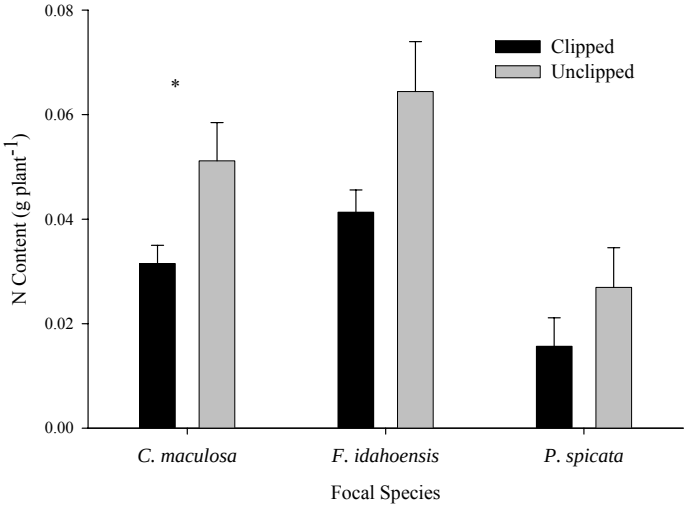

Figure 23. Clipping effects on N content of each species. *C. maculosa* plants were grown with all neighbors. Error bars represent one standard error of the mean. (*) Indicates significant difference between means of AM vs. non-AM treatments ($\alpha<0.05$).

Plant Phosphorous

Plant P concentrations were 0.17% ± 0.009, 0.17% ± 0.006 and 0.19% ± 0.008. No significant effects of AM or clipping on P concentration were present for any species grown with a *C. maculosa* neighbor (Table 22). For *C. maculosa* plants with all neighbors, the clipping treatment influenced P concentration (Table 23), with higher P concentration in clipped plants (Figure 24).

Table 22. Results of ANOVA for P concentration. All plants were grown with a *C. maculosa* neighbor. Data were not transformed for any species

		C. maculosa		*F. idahoensis*		*P. spicata*	
Factor	Df	F	p	F	p	F	p
AM	1	1.29	0.289	0.48	0.508	0.01	0.928
Clipping	1	2.39	0.160	2.33	0.166	0.08	0.788
AM X Clipping	2	0.86	0.380	0.94	0.360	0.08	0.788
Residuals	8						

Table 23. Results of ANOVA for *C. maculosa* P concentration with all neighbors (g). Data were not transformed.

Factor	Df	F	p
AM	1	3.19	0.087
Neighbor	2	1.13	0.341
Clipping	1	15.09	0.001
AM X Neighbor	2	0.11	0.896
AM X Clipping	2	0.00	0.972
Neighbor X Clipping	4	0.28	0.758
AM X Neighbor X Clipping	4	3.32	0.054
Residuals	23		

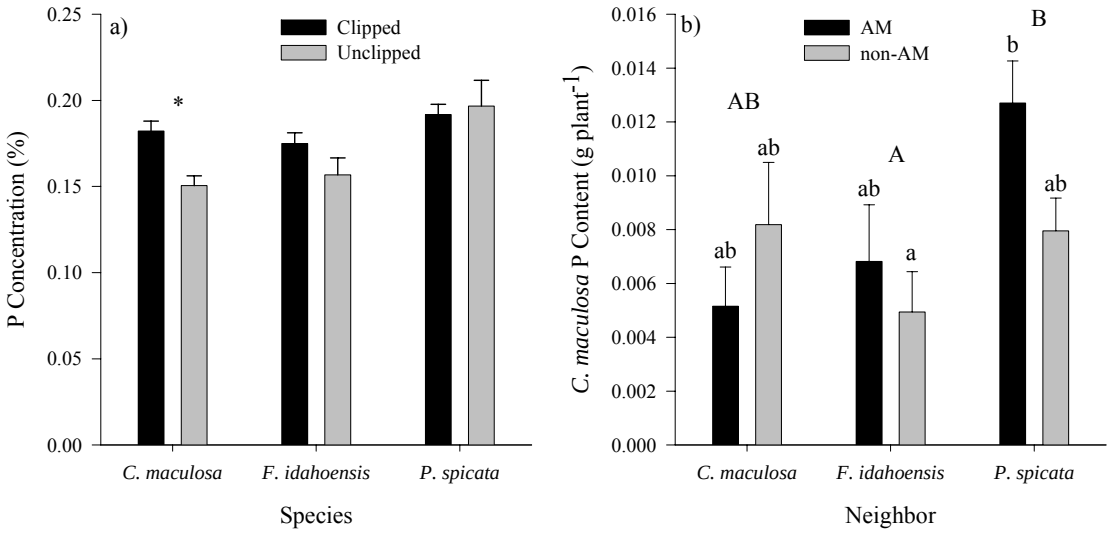

Figure 24. a) Clipping effects on P concentration. Grass species were grown with a *C. maculosa* neighbor only, and *C. maculosa* was grown with all neighbor species. (*) Indicates significant difference between means of AM vs. non-AM treatments ($\alpha<0.05$). b) Neighbor and AM effects on *C. maculosa* P content. Error bars represent one standard error of the mean. Lowercase letters represent significant differences between treatments within each neighbor species and capital letters represent significant differences between neighbors ($\alpha<0.05$).

Plant P content of each species grown with a *C. maculosa* neighbor was also not affected by AM or clipping treatments (Table 24). For *C. maculosa* plants with all

neighbors, P content was affected by neighbor species (Table 25). Phosphorous content was greatest in plants with a *P. spicata* neighbor, and lowest in plants with a *C. maculosa* neighbor (Figure 24b). *Centaurea maculosa* was affected by the clipping treatment (Table 25) and had higher P content in unclipped plants (Table 26). Although not significant, an interaction of AM and neighbor factors influenced *C. maculosa* P content (Table 25). Phosphorous content tended to be higher with AM when paired with grass species and lower with AM paired with a *C. maculosa* neighbor (Figure 24b).

Table 24. Results of ANOVA for P content. All plants were grown with a *C. maculosa* neighbor. Data were not transformed for any species

		C. maculosa		*F. idahoensis*		*P. spicata*	
Factor	Df	F	p	F	p	F	p
AM	1	1.11	0.322	0.17	0.691	0.03	0.866
Clipping	1	0.85	0.384	2.68	0.140	0.91	0.372
AM X Clipping	2	0.19	0.674	2.52	0.151	0.24	0.638
Residual Df		8		8		7	

Table 25. Results of ANOVA for *C. maculosa* P content with all neighbors (g). Data were not transformed.

Factor	Df	F	p
AM	1	0.82	0.375
Neighbor	2	4.24	0.026
Clipping	1	7.45	0.012
AM X Neighbor	2	2.92	0.073
AM X Clipping	2	0.34	0.564
Neighbor X Clipping	4	0.19	0.825
AM X Neighbor X Clipping	4	0.88	0.427
Residuals	24		

Table 26. Plant P content for each species by clipping treatment. *Centaurea maculosa* was grown with all neighbor species.

	Clipping treatment	Mean P content (mg plant^{-1})
C. maculosa	Unclipped	9.44 ± 1.31
	Clipped	5.81 ± 0.68
F. idahoensis	Unclipped	13.50 ± 2.37
	Clipped	9.42 ± 1.00
P. spicata	Unclipped	6.82 ± 2.04
	Clipped	4.10 ± 1.49

Discussion

AM influence on competition

Arbuscular mycorrhizal fungi can influence competition between native and invasive plants by altering patterns of nutrient uptake and allocation. Plants generally benefit from the additional nutrients acquired through mycorrhizal symbiosis, especially in low nutrient environments, such as the semi-arid rangelands invaded by *C. maculosa*. However factors such as competition from neighbors and herbivory make the effect of the symbiont on the host more difficult to predict. A potential tradeoff results between the allocation of carbon to AM fungi, which results in increased nutrient acquisition, versus allocation of carbon to regrowth of photosynthetic tissue.

For plants that were not clipped in this experiment, AM fungi did not influence competition as measured by relative growth. *Centaurea maculosa* plants were 39% of the total pot biomass under AM conditions and 28% of the biomass without AM fungi but with high variance in biomass between pots, these numbers were not significantly different. In other research, *C. maculosa* biomass is significantly larger when grown with

a *F. idahoensis* neighbor in the presence of AM fungi, and *F. idahoensis* plants are significantly smaller (Marler *et al.* 1999a). In my experiment, *C. maculosa* and *F. idahoensis* plants were the same size grown together with and without AM fungi.

AM and Herbivory

The effect of AM fungi on response to herbivory has been mixed in previous studies, likely due to differences in experimental conditions, including species of plant and fungi, and nutrient status of plants. Arbuscular mycorrhizal fungi may be detrimental to regrowth if photosynthate is allocated to AM fungi instead of new shoot tissue.

When *C. maculosa* plants were clipped, AM fungi affected regrowth with either another *C. maculosa* or a *F. idahoensis* neighbor. With a *C. maculosa* neighbor, greater compensatory growth was observed in the AM treatment than the non-AM treatment. In contrast, when grown with an *F. idahoensis* neighbor, clipped plants had 38% of the biomass of unclipped plants under AM conditions, and 97% of unclipped plants under non-AM conditions, indicating greater compensatory growth without AM. The carbon cost of AM could have limited regrowth of *C. maculosa* under AM conditions with a *F. idahoensis* neighbor, however this does not explain why compensatory growth occurred with a *C. maculosa* neighbor under AM conditions. Alternatively, since the biomass of *F. idahoensis* tended to increase when *C. maculosa* neighbors were clipped with AM fungi present, the additional growth of *F. idahoensis* could have suppressed *C. maculosa* regrowth. While my results indicate that *F. idahoensis* experienced release from competition when *C. maculosa* was clipped with AM fungi present, the opposite

trend occurred without AM fungi, where *F. idahoensis* tended to be smaller (p=0.025) when *C. maculosa* was clipped . An additional advantage of AM fungi to *F. idahoensis* plants was that compensatory growth occurred in this plant only with AM fungi.

Centaurea maculosa biomass did not have compensatory growth with a *P. spicata* neighbor for either AM treatment. *Pseudoroegneria spicata* neighbors, however, compensated for clipping either with or without AM fungi. These results could be confounded, however, by the large size discrepancy between these two species. Growing with *P. spicata*, *C. maculosa* plants are among the largest plants in the experiment, whereas *P. spicata* plants are among the smallest

Clipping effect on AM fungi

Clipped *F. idahoensis* plants had lower colonization than unclipped plants. Lower colonization after clipping could occur because less carbon is available for the symbiosis following clipping. With less photosynthetic tissue, plants may allocate carbon to regrowth instead of to mycorrhizal symbiosis (Borowicz & Fitter 1990). Clipping the neighboring *C. maculosa* plant also reduced AM colonization of *F. idahoensis.* In Chapter 2, *F. idahoensis* plants grown with highly colonized *C. maculosa* plants had higher colonization than plants grown alone. I suggested that ERH from *C. maculosa* plants could have colonized *F. idahoensis*. The decrease in *F. idahoensis* colonization with *C. maculosa* clipping in this study may be the result of reduced carbon allocation by *C. maculosa* to its fungal symbiont. These results suggest

that *C. maculosa* may donate more carbon to a common ERH network than *F. idahoensis*.

The measure of AM fungi external to roots (ERH) was not affected by clipping treatment, similar to results of (Walling & Zabinski 2005). The measure of ERH, however, does not differentiate between live and dead hyphae, so I may not have detected a decrease in live hyphae after clipping. Extraradical hyphal density also did not differ by species, as seen by Walling and Zabinski (2004) and in Chapter 2. *Centaurea maculosa* had the highest ERH density in both of these studies. In Chapter 2, ERH hyphal density was greater in *F. idahoensis* pots which had a *C. maculosa* neighbor than those with a grass neighbor or growing alone. All plants in this experiment were grown with *C. maculosa* neighbors, which could partially explain the high ERH density present in all species in this experiment.

Nutrients

Centaurea maculosa was the only plant with higher nutrient concentrations after clipping, which could be due to an increase in N-rich defense chemicals following herbivory. Alternatively, the higher concentration may be due to similar quantities of N in smaller (clipped) plants versus larger (unclipped) plants. Native grasses had more compensatory growth than *C. maculosa*, indicating that newly acquired resources were allocated to regrowth for these species, and that new tissue had comparable nutrient concentrations to tissues that were clipped.

Summary

The relative abilities of weedy species and native neighbors to compensate for herbivory influences the effectiveness of herbivory as a weed control method (Olson 1999). This experiment provides evidence that partial removal of *C. maculosa* aboveground biomass provides some release of competition for neighboring grasses. As opposed to other studies with root herbivory (Steinger & Muller-Scharer 1992) and shoot herbivory (Newingham & Callaway),(Callaway *et al.* 1999) there was no evidence from this study that *C. maculosa* grew better than native grasses following herbivory. The level of 75% biomass removal reduced *C. maculosa* biomass enough to affect its competitive interactions with neighbor species. Other experiments with insect root and shoot herbivory had lower levels of tissue removal (Callaway *et al.* 1999), which may explain why these experiments demonstrate increased growth of *C. maculosa* following herbivory. The lack of complete compensation for herbivory observed for some cases in this study could be due to the severity of defoliation (Alward & Joern 1993). Newingham and Callaway (In Review) found that when shoot tissue removal is lower than 40%, *C. maculosa* plants were 9% smaller than unaffected plants, whereas when tissue loss was greater than 40%, regrowth resulted in plants that were 58% smaller than unaffected plants.

Arbuscular mycorrhizae did not influence competitive interaction between native grasses and *C. maculosa* as seen in previous studies, but it did influence regrowth with some neighbors. Under AM conditions, *C. maculosa* only had compensatory growth with a conspecific. Since AM colonization is common in the field, the response to

herbivory in AM treatments is more predictive for field conditions and for informing management. These results indicate that grazing may be an important tool for managing the spread of *C. maculosa*, but field trials varying both grazing intensity and with plant communities that vary in composition are necessary.

4. CONCLUSIONS

Arbuscular mycorrhizal function

The AM symbiosis can positively influence plant nutrition and growth, however host plant benefit depends on soil nutrients, the fungal species, and the plant species involved in the symbiosis (Johnson *et al.* 1997). In Chapter 2 of this research, AM fungi had a negative effect on plant growth (39-50% reduction in AM versus non-AM plants) while in Chapter 3 there was no effect of AM on host plant biomass. This difference in response may be due to the method of inoculation used in the two studies, differences in soils, or differences in growing conditions. Soil was collected during two different years from the same location and plants were grown in the same size pots with similar greenhouse conditions. Both experiments were allowed to grow approximately four months during high light availability of the summer months. Soil P was similar between the two soil mediums (3.3mg/kg and 4.3mg/kg for Chapter 2 and Chapter 3, respectively), while soil NO_3 levels may have been higher in chapter 3 (4.9mg/kg and 9.7mg/kg for Chapter 2 and Chapter 3, respectively). These soil NO_3 levels are both still considered low for field conditions.

The method of inoculum differed slightly. For the experiment in Chapter 2, field soil was mixed into the soil medium (8:1, sand:soil) and the entire soil medium for the non-AM treatment was pasteurized, similar to methods in Walling and Zabinski (2005). For the experiment in Chapter 3, the AM inoculum was added to pots as a 1.5 cm layer of unpasteurized field soil 4 cm below the surface, or a pasteurized layer for the non-AM treatment. The proportion of inoculum soil was similar for both methods, so inoculum

quantity should have been consistent. Colonization levels were also similar between the two studies (18, 3 and 7% for the three species in Chapter 2 vs. 18, 9, and 6.5% in Chapter 3), but more variation was seen in colonization levels in Chapter 3. Extraradical hyphal length density above the background level, however, tended to be greater in the second experiment (0.7 m g^{-1} soil in Chapter 2 and 1.6 m g^{-1} soil in Chapter 3 for *C. maculosa* plants grown with a conspecific)

It is possible that with higher inoculum density close to seedling roots, plants were colonized by AM fungi more quickly with the banding method, where inoculum was added as a layer, than with the inoculum mixed throughout the pot. If plants form mycorrhizae earlier in their growth, they may be more protected from the negative effects of pathogenic fungi present in the AM treatment soil (Newsham *et al.* 1995). Since AM fungi are obligate symbionts, propagules would not increase in density until a plant is colonized, whereas pathogenic fungi may have increased to a point that limited plant growth in pots with unpasteurized field soil inoculum. The microbial wash added to the non-AM soil was intended to re-introduce soil biota including bacteria and pathogenic fungi, but some pathogenic fungi are larger than the pore size used to filter the microbial wash and would not have been reintroduced.

Plant Competition

The experiment in Chapter 2 was designed to test hypotheses relating to mechanisms of AM influence on competition between *C. maculosa* and *F. idahoensis,* specifically increased production of extraradical hyphae (Walling & Zabinski 2004; Walling & Zabinski 2005) and enhanced nutrient uptake (Zabinski *et al.* 2002).

Arbuscular mycorrhizal fungi, however, did not appear to influence competition between these two species in Chapter 2, instead, the potential for root contact was important. I found similar results for *C. maculosa* and *P. spicata* species combinations. It was also difficult to compare across AM treatments since AM plants were much smaller than non-AM plants in Chapter 2.

Festuca idahoensis plants grown in the experiment in Chapter 2, and in the experiment of Walling and Zabinski (2005), were very small compared to those in Chapter 3 (0.9g vs. 7.5g). Biomass has a large effect on the outcome of plant competition (Bonser & Reader 1995). For the experiment reported in Chapter 3, *F. idahoensis* seeds were purchased from Wind River Seeds. The variety was *F. idahoensis* var. *winchester*, which has a more robust growth form and a darker green color than the *F. idahoensis* used in previous studies or found in the field. *Festuca idahoensis* plants were also seeded 23 days prior to *P. spicata* or *C. maculosa* plants.

While *C. maculosa* was the largest plant in Chapter 2, *F. idahoensis* was the largest plant in Chapter 3. *Centaurea maculosa* is more plastic in terms of allocation to roots, in that it has a lower RMR with AM fungi while the grasses did not. This would benefit a plant with a small neighbor such as *F. idahoensis* used in Chapter 2, but with a large neighbor the costs of reduced root competitive ability could have outweighed the benefits of increased nutrient acquisition from AM fungi.

AM and clipping interactions

In Chapter 3, AM fungi influenced plant response to herbivory, and the effect depended on neighbor species. Unclipped *C. maculosa* plants were always larger than

clipped plants grown with a *P. spicata* neighbor. *Centuarea maculosa* plants with a *F. idahoensis* neighbor compensated for herbivory only without AM fungi present. When *C. maculosa* was grown with a conspecific, however, clipped plants compensated for herbivory only with AM fungi present. Arbuscular mycorrhizal fungi likely aided regrowth of biomass through increased nutrient acquisition when *C. maculosa* had intraspecific competition, but with intense interspecific competition with a large *F. idahoensis*, competition for nutrients reduced *C. maculosa's* ability to regrow.

For the *F. idahoensis*-*C. maculosa* species combination, *F. idahoensis* achieved more benefit from the symbiosis in terms of growth. *Centaurea maculosa* benefited more from AM in Chapter 2, while *F. idahoensis* was larger and benefited more from AM in Chapter 3. It is unclear whether this is a difference between species or if simply larger plants benefit more from the symbiosis because of relatively less carbon cost given the greater photosynthetic area.

The amount of regrowth of grass species in Chapter 3 was dependent on the presence of AM fungi for *F. idahoensis* and was not affected by AM fungi for *P. spicata*. Both AM and non-AM *P. spicata* plants compensated for herbivory after four weeks.

Herbivory had direct and indirect effects on AM colonization of *F. idahoensis.* Colonization was lower in clipped plants than unclipped plants, and was also lower than unclipped plants when the neighboring *C. maculosa* plant was clipped. With the findings in Chapter 2 that colonization of *F. idahoensis* increased when grown with *C. maculosa*, I suspect that for unclipped plants ERH from *C. maculosa* colonized neighboring *F. idahoensis* plants. When *C. maculosa* plants are clipped, photosynthate may be re-

allocated away from extraradical hyphae production, hence less colonization of neighbors occurs.

Implications for management of *Centaurea maculosa*

Chapter 2 of this research identified potential traits of *C. maculosa* which may lead to increased competitive ability of *C. maculosa*. This species had higher ERH production than native grasses and likely even influenced colonization of *F. idahoensis* neighbors. Walling and Zabinski (2005) also found greater ERH density in *C. maculosa* pots, Zabinski *et al.* (2002) suggest that increased P uptake via ERH may explain *C. maculosa*'s increased competitive ability when grown with *F. idahoensis*. I did not see increased competitive ability with AM fungi in this research, possibly because N, not P was more limiting to plants in both Chapters 2 and 3. Presumably because *C. maculosa* had higher AM colonization and ERH density than native grasses, it also had higher nutrient concentrations of both N and P with AM fungi present. *Centaurea maculosa* was also the only species to have higher N concentration with AM fungi in Chapter 3.

Chapter 3 investigated whether shoot herbivory of *C. maculosa* is an effective weed management tool in terms of damage to *C. maculosa* and benefit to neighboring native grasses. *C. maculosa* was only able to regrow after clipping in 2 of the 6 AM and neighbor species combinations. These were grown with another *C. maculosa* plant and with AM fungi, and grown with a *F. idahoensis* neighbor without AM fungi. The lack of compensatory response in some cases could be due to the severity of defoliation (75% of aboveground biomass removal) as opposed to other studies with *C. maculosa* where compensation occurred and increased competitive ability of *C. maculosa* occurred

following defoliation (Ridenour & Callaway 2003). Results of this research indicate that neighbor species and identity are important considerations for predicting the outcome of herbivory in the field and the use of grazing to control *C. maculosa*. Arbuscular mycorrhizal fungi also influenced response to herbivory in this study for some cases and should be considered when predicting the effects of grazing, due to its prevalence in rangeland ecosystems.

REFERENCES CITED

Aerts R., Boot R.G.A. & Vanderaart P.J.M. 1991. The relation between aboveground and belowground biomass allocation patterns and competitive ability. *Oecologia* 87, 551-559.

Aerts R. & Chapin F.S. 2000. The mineral nutrition of wild plants revisited: A re-evaluation of processes and patterns. *Advances in Ecological Research* 30, 1-67.

Allen E.B. & Allen M.F. 1984. Competition between plants of different successional stages: mycorrhizae as regulators. *Canadian Journal of Botany* 62, 2625-2629.

Allen E.B. & Allen M.F. 1986. Water relations of xeric grasses in the field - interactions of mycorrhizas and competition. *New Phytologist* 104, 559-571.

Allen E.B. & Allen M.F. 1990. The mediation of competition by mycorrhizae in successional and patchy environments. In: *Perspectives on Plant Competition* eds Grace J.B. & Tilman D. pp. 367-389. Academic Press, Inc., San Diego.

Alward R.D. & Joern A. 1993. Plasticity and overcompensation in grass responses to herbivory. *Oecologia* 95, 358-364.

Bais H.P., Vepachedu R., Gilroy S., Callaway R.M. & Vivanco J.M. 2003. Allelopathy and exotic plant invasion: From molecules and genes to species interactions. *Science* 301, 1377-1380.

Baucom R.S. & Mauricio R. 2004. Fitness costs and benefits of novel herbicide tolerance in a noxious weed. *Proceedings of the National Academy of Sciences of the United States of America* 101, 13386-13390.

Bethlenfalvay G.J., Evans R.A. & Lesperance A.L. 1985. Mycorrhizal colonization of crested wheatgrass as influenced by grazing. *Agronomy Journal* 77, 233-236.

Bever J.D. & Dakessian S. 1984. Grazing effects on mycorrhizal colonization and floristic composition of the vegetation on a semiarid range in northern Nevada. *Journal of Range Management* 37, 312-316.

Bever J.D., Pringle A. & Schultz P.A. 2002. Dynamics within the plant - arbuscular mycorrhizal fungal mutualism: testing the nature of community feedback. In: *Mycorrhizal Ecology* eds van der Heijden M.G.A. & Sanders I.R. pp. 267-292. Springer-Verlag, Berlin Heidelberg.

Bever J.D., Westover K.M. & Antonovics J. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology* 85, 561-573.

Bidartondo M.I., Redecker D., Hijri I., Wiemken A., Bruns T.D., Dominguez L., Sersic A., Leake J.R. & Read D.J. 2002. Epiparasitic plants specialized on arbuscular mycorrhizal fungi. *Nature* 419, 389-392.

Blicker P.S., Olson B.E. & Engel R.S. 2002. Traits of the invasive *Centaurea maculosa* and two native grasses: effect of N supply. *Plant and Soil* 247, 261-269.

Boggs K.W. & Story J.M. 1987. The population age structure of spotted knapweed (Centaurea maculosa) in Montana. *Weed Science* 35, 194-198.

Bonser S.P. & Reader R.J. 1995. Plant competition and herbivory in relation to vegetation biomass. *Ecology* 76, 2176-2183.

Borowicz V.A. & Fitter A.H. 1990. Effects of endomycorrhizal infection, artificial herbivory, and parental cross on growth of *Lotus corniculatus* L. *Oecologia* 82, 402-407.

Briske D.D. & Richards J.H. 1994. Physiological responses of individual plants to grazing: current status and ecological significance. In: *Ecological implications of livestock herbivory in the west* eds Laycock W.A. & Pieper R.D. 1st ed. pp. 147-176. Society for Range Management, Denver.

Callaway R.M., DeLuca T.H. & Belliveau W.M. 1999. Biological-control herbivores may increase competitive ability of the noxious weed *Centaurea maculosa*. *Ecology* 80, 1196-1201.

Callaway R.M., Thelen G.C., Rodriguez A. & Holben W.E. 2004. Soil biota and exotic plant invasion. *Nature* 427, 721-723.

Carey E.V., Marler M.J. & Callaway R.M. 2004. Mycorrhizae transfer carbon from a native grass to an invasive weed: evidence from stable isotopes and physiology. *Plant Ecology* 172, 133-141.

Casper B.B. & Jackson R.B. 1997. Plant competition underground. *Annual Review of Ecology and Systematics* 28, 545-570.

Chapin F.S. & McNaughton S.J. 1989. Lack of compensatory growth under phosphorus deficiency in grazing-adapted grasses from the Serengeti plains. *Oecologia* 79, 551-557.

Crawley M.J. 1997. Plant-Herbivore Dynamics. In: *Plant Ecology* [ed.] Crawley M.J. 2nd ed. pp. 51-72. Cambridge University Press, Cambridge.

Daft M.J. & El-Giahmi A.A. 1978. Effect of arbuscular mycorrhiza on plant growth VIII. Effects of defoliation and light on selected hosts. *New Phytologist* 80, 365-372.

Del Vecchio T.A., Gehring C.A., Cobb N.S. & Whitham T.G. 1993. Negative effects of scale insect AM-Herbivory on the ectomycorrhizae of juvenile pinyon pine. *Ecology* 74, 2297-2302.

Eom A.-H., Wilson G.T. & Hartnett D.C. 2001. Effects of ungulate grazers on arbuscular mycorrhizal symbiosis and fungal community structure in tallgrass prairie. *Mycologia* 93, 233-242.

Falik O., Reides P., Gersani M. & Novoplansky A. 2003. Self/non-self discrimination in roots. *Journal of Ecology* 91, 525-531.

Fitter A. 1997. Nutrient Acquisition. In: *Plant Ecology* [ed.] Crawley M.J., 2nd ed. pp. 51-72. Cambridge University Press, Cambridge.

Fitter A.H. 1977. Influence of mycorrhizal infection on competition for phosphorus and potassium by two grasses. *New Phytologist* 12, 406-412.

Frey B. & Schüepp H. 1993. Acquisition of nitrogen by external hyphae of arbuscular mycorrhizal fungi associated with *Zea mays* L. *New Phytologist* 124, 221-230.

Gange A.C. & Ayres R.L. 1999. On the relation between arbuscular mycorrhizal colonization and plant "benefit". *Oikos* 87, 615-621.

Gehring C.A. & Whitham T.G. 1994. Interactions between aboveground herbivores and the mycorrhizal mutualists of plants. *Trends in Ecology & Evolution* 9, 251-255.

Goldberg D.E. 1990. Components of resource competition in plant communities. In: *Perspectives on Plant Competition* eds Grace J.B. & Tilman D. pp. 367-389. Academic Press, Inc., San Diego.

Grime J.P., Mackey J.M.L., Hillier S.H. & Read D.J. 1987. Floristic diversity in a model system using experimental microcosms. *Nature* 328, 420-422.

Hart M.M., Reader R.J. & Klironomos J.N. 2003. Plant coexistence mediated by arbuscular mycorrhizal fungi. *Trends in Ecology & Evolution* 18, 418-423.

Hartnett D.C., Hetrick B.A.D., Wilson G.W.T. & Gibson D.J. 1993. Mycorrhizal influence on intra- and interspecific neighbor interactions amount co-occurring prairie grasses. *Journal of Ecology* 81, 787-795.

Hartnett D.C. & Wilson G.T. 2002. The role of mycorrhizas in plant community structure and dynamics: lessons from grasslands. *Plant and Soil* 244, 319-331.

Hetrick B.A.D., Hartnett D.C., Wilson G.W.T. & Gibson D.J. 1994. Effects of mycorrhizae, phosphorus availability, and plant density on yield relationships among competing tallgrass prarie grasses. *Canadian Journal of Botany* 72, 168-176.

Hodge A. 2000. Microbial ecology of the arbuscular mycorrhiza. *FEMS Microbiology Ecology* 32, 91-96.

Jakobsen I., Abbott L.K. & Robson A.D. 1992. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. *New Phytologist* 120, 371-380.

Jakobsen I., Gazey C. & Abbott L.K. 2001. Phosphate transport by communities of arbuscular mycorrhizal fungi in intact soil cores. *New Phytologist* 149, 95-103.

Jakobsen I., Smith S.E. & Smith F.A. 2002. Function and diversity of arbuscular mycorrhizae in carbon and mineral nutrition. In: *Mycorrhizal Ecology* eds van der Heijden M.G.A. & Sanders I.R. pp. 75-92.

Johnson N.C., Graham J.H. & Smith F.A. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist* 135, 575-586.

Keane R.M. & Crawley M.J. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* 17, 164-170.

Klironomos J.N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* 417, 67-70.

Klironomos J.N. 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology* 84, 2292-2301.

Koerselman W. & Meuleman A.F.M. 1996. The vegetation N:P ratio: A new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology* 33, 1441-1450.

Koide R.T. & Elliott G. 1989. Cost, benefit and efficiency of the vesicular-arbuscular mycorrhizal symbiosis. *Functional Ecology* 3, 252-255.

Koide R.T. & Li M.G. 1989. Appropriate controls for vesicular arbuscular mycorrhiza research. *New Phytologist* 111, 35-44.

Kula A.A.R., Hartnett D.C. & Wilson G.W.T. 2005. Effects of mycorrhizal symbiosis on tallgrass prairie plant-herbivore interactions. *Ecology Letters* 8, 61-69.

LeJeune K.D. & Seastedt T.R. 2001. *Centaurea* species: the forb that won the west. *Conservation Biology* 15, 1568-1574.

Marler M.J., Zabinski C.A. & Callaway R.M. 1999a. Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. *Ecology* 80, 1180-1186.

Marler M.J., Zabinski C.A., Wojtowicz T. & Callaway R.M. 1999b. Mycorrhizae and fine root dynamics of *Centaurea maculosa* and native bunchgrasses in western Montana. *Northwest Science* 73, 217-224.

Marschner H. 1995. Nutrient availability in soils. In: *Mineral Nutrition of Higher Plants*, 2 ed. pp. 483-507. Academic Press, London.

Maschinski J. & Whitham T.G. 1989. The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. *The American Naturalist* 134, 1-19.

McConnaughay K.D.M. & Bazzaz F.A. 1991. Is physical space a soil resource? *Ecology* 72, 94-103.

McGonigle T.P., Miller M.H., Evans D.G., Fairchild G.L. & Swan J.A. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* 115, 495-501.

McNaughton S.J. 1983. Compensatory plant growth as a response to herbivory. *Oikos* 40, 329-336.

Miller R.M., Reinhardt D.R. & Jastrow J.D. 1995. External hyphal production of vesicular-arbuscular mycorrhizal fungi in pasture and tallgrass prairie communities. *Oecologia* 103, 17-23.

Moora M. & Zobel M. 1998. Can arbuscular mycorrhiza change the effect of root competition between conspecific plants of different ages? *Canadian Journal of Botany* 76, 613-619.

Mosse B. 1959. Observations on the extra-matrical mycelium of a vesicular-arbuscular endophyte. *Transactions of the British Mycological Society* 42, 439-448.

Newingham B.A. & Callaway R.M. In Review. Aboveground herbivory on the invasive plant, *Centaurea maculosa*, does not reduce its competitive effects on conspecifics and neighbors. *Oikos.*

Newsham K.K., Fitter A.H. & Watkinson A.R. 1995. Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *Journal of Ecology* 83, 991-1000.

Nicolson T.H. 1959. Mycorrhiza in the Gramineae. *Transactions of the British Mycological Society* 42, 421-438.

Olson B.E. 1999a. Grazing and Weeds. In: *The biology and management of rangeland weeds*, pp. 85-96.

Olson B.E. 1999b. Impacts of noxious weeds on ecologic and economic systems. In: *The biology and management of rangeland weeds* eds Sheley R.L. & Petroff J.K.

Olson B.E. & Wallander R.T. 2001. Sheep grazing spotted knapweed and Idaho fescue. *Journal of Range Management* 54, 25-30.

Phillips J.M. & Hayman D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* 55, 158-161.

R Development Core Team. 2004. R: A language and environment for statistical computing. 2.0.1. R Foundation for Statistical Computing. Vienna, Austria. ISBN 3-900051-07-0. URL http://www.R-project.org.

Ridenour W.L. & Callaway R.M. 2003. Root herbivores, pathogenic fungi, and competition between Centaurea maculosa and Festuca idahoensis. *Plant Ecology* 169, 161-170.

Shumway D.L. & Koide R. 1994. Reproductive responses to mycorrhizal colonization of *Abutilon theophrasti* Medic. plants grown for two generations on the field. *New Phytologist* 128, 219-224.

Simard S.W., Perry D.A., Jones M.D., Myrold D.D., Durall D.M. & Molina R. 1997. Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* 388, 579-582.

Smith S.E. & Read D.J. 1997. *Mycorrhizal Symbiosis*, 2nd edn. Academic Press, San Diego.

Steinger T. & Muller-Scharer H. 1992. Physiological and growth responses of Centaurea maculosa (Asteraceae) to root herbivory under varying levels of interspecific plant competition and soil nitrogen availability. *Oecologia* 91, 141-149.

Strauss S.Y. & Agrawal A.A. 1999. The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology & Evolution* 14, 179-185.

Sylvia D.M. 1992. Quantification of external hyphae of vesicular-arbuscular mycorrhizal fungi. In: *Methods in microbiology*, pp. 54-65. Academic Press.

Tennant D. 1975. A test of a modified line intersect method of estimating root length. *Journal of Ecology* 63, 995-1001.

Thelen G.C., Vivanco J.M., Newingham B., Good W., Bais H.P., Landres P., Caesar A. & Callaway R.M. 2005. Insect herbivory stimulates allelopathic exudation by an invasive plant and the suppression of natives. *Ecology Letters* 8, 209-217.

Thompson J.P. 1990. Soil sterilization methods to show VA-mycorrhizae aid P and Zn nutrition of wheat in vertisols. *Soil Biology and Biochemistry* 22, 229-240.

Tilman D. 1997. Mechanisms of Plant Competition. In: *Plant Ecology* [ed.] Crawley M.J., 2nd ed. pp. 51-72. Cambridge University Press, Cambridge.

Trent J.D., Wallace L.L., Svejcar T.J. & Christiansen S. 1988. Effect of grazing on growth, carbohydrate pools, and mycorrhizae in winter wheat. *Canadian Journal of Plant Science* 68, 115-120.

Turner C.L., Seastedt T.R. & Dyer M.I. 1993. Maximization of aboveground grassland production - the role of defoliation frequency, intensity and history. *Ecological Applications* 3, 175-186.

Tyser R.W. & Key C.H. 1988. Spotted knapweed in natural area fescue grasslands: An ecological assessment. *Northwest Science* 62, 151-160.

Wallace L.L. 1981. Growth, morphology and gas exchange of mycorrhizal and nonmycorrhizal *Panicum coloratum* L., a C4 grass species, under different clipping and fertilization regimes. *Oecologia* 49, 272-278.

Wallace L.L. 1987. Mycorrhizas in grasslands: Interactions of ungulates, fungi and drought. *New Phytologist* 105, 619-632.

Walling S.Z. & Zabinski C.A. 2004. Host plant differences in arbuscular mycorrhizae: Extra radical hyphae differences between an invasive forb and a native bunchgrass. *Plant and Soil* 265, 335-344.

Walling S.Z. & Zabinski C.A. 2005. Defoliation effects on arbuscular mycorrhizae and plant growth of two native bunchgrasses and an invasive forb. *Applied Soil Ecology*

Watson A.K. & Renney A.J. 1974. The biology of Canadian weeds *Centaurea diffusa* and *C. maculosa*. *Canadian Journal of Plant Science* 54, 687-701.

Zabinski C.A., Quinn L. & Callaway R.M. 2002. Phosphorous uptake, not carbon transfer, explains arbuscular mycorrhizal enhancement of *Centaurea maculosa* in the presence of native grassland species. *Functional Ecology* 16, 758-765.