

University of Nevada, Reno

**Investigations of physiological and competitive relationships of *Elymus*
species related to establishment in the Great Basin, USA.**

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in
Natural Resources and Environmental Science

By

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Abstract

The Great Basin of western North America is rapidly being invaded by exotic annual grasses that decrease cover of native perennial bunchgrasses and shrubs. *Bromus tectorum* (cheatgrass) is the most widespread and problematic of the annual grass invaders in the Great Basin. Restoration in the Great Basin has had limited success. Restoration success could be related to physiology of native plants in competition for limiting nutrients: *Bromus tectorum* employs ruderal strategies of resource acquisition, is an effective competitor for water and nitrogen, and can cause mortality of natives in invaded fields as well as prevent establishment of native seedlings. In response to the survival pressure placed on native communities by invaders, natives may experience evolutionary changes. Directional selection for plant traits in restored populations may occur as a result of differential mortality related to the ability to acquire resources. The goal of this research was to determine what physiological traits of native perennial grasses lead to successful establishment under *Bromus tectorum* competition and to determine if selection for root traits is occurring during restoration. Identification of key plant traits related to establishment and competition is an important step in selecting and breeding plant materials with the best chance of success in restoration. Traits selected during establishment of natives under competition with *Bromus tectorum* may give insight into the mechanisms of plant establishment. We focus on *Elymus elymoides* and *Elymus multisetus* because they have wide geographic ranges and have been identified as good competitors against *Bromus tectorum*.

Results from the first study indicate that different *Elymus* seed sources had few differences in their physiology and resource acquisition. *Bromus tectorum* dominated soil water

use and nutrient acquisition at the *Elymus* seedling stage, but was much less effective in competition with mature *Elymus* plants. Competitive effect of mature *Elymus* plants on *Bromus tectorum* was related to water acquisition. Results from the second study show that directional selection for smaller plants with a higher fraction of root biomass occurred in two sites in the Great Basin during seeding of *Elymus* plants. Together, these results are consistent with selection for traits that allow plants to survive as seedlings in areas of high *Bromus tectorum* cover, but not consistent with selection for nutrient acquisition or competitive ability with *Bromus tectorum*. Plant selection for effective restoration of arid rangelands should focus on selecting seedlings for establishment under *Bromus tectorum* pressure and mature plants capable of inducing competitive effect through water acquisition.

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Introduction

The Great Basin of western North America is an arid to semi-arid region and is experiencing rapid invasion by exotic annual grasses that are gaining dominance and decreasing cover of native perennial bunchgrasses and shrubs (Wisdom & Chambers 2009; Young 1992). *Bromus tectorum* was introduced into the western U.S.A. in the late 1800's (Pellant, Abbey & Karl 2004) and now occupies over 40,000 km² in the states of Nevada and Utah alone (Bradley & Mustard 2006). Annual grass invasion increases fire frequency and alters ecosystem services (D'Antonio & Vitousek 1992), including hydrological relationships and nutrient cycling (Knapp 1996; Mack 1981). Dominance of invasive annual plants is related to increased disturbance from grazing and increased fire frequency; intact ecosystems with mature plants are relatively resistant to invasion (Chambers *et al.* 2007). Restoration of Great Basin ecosystems has proven difficult due to limited precipitation and is further complicated by the presence of invasive species and continued disturbance.

Restoration success could be related to physiology of native plants in competition for limiting nutrients. Water and nitrogen limit productivity in arid lands (Smith & Nowak 1990), and acquisition of water and nitrogen depends on spatial and temporal deployment of roots (Evans *et al.* 1970; Eissenstat & Caldwell 1988; Harris & Wilson, 1967). Physiological aspects of root traits that allow plants to access limiting nutrients can help plant establishment. Identification of key plant traits related to

resource acquisition is an important step in finding plant restoration materials with the highest probability for success in *Bromus tectorum* invaded ecosystems of the Western U.S.

Species interactions are drivers of rapid co-evolutionary processes (Thompson 1999). In response to the survival pressure placed on native communities by invaders, natives may experience evolutionary changes (Kinnison & Hairston 2007; Strauss, Lau & Carroll 2006; Mooney & Cleland, 2001). *Bromus tectorum* has a large effect on the nitrogen (Blank & Young, 2004) and water status of invaded systems (Cline *et al.* 1977). Traits that allow native plants to compete with *Bromus tectorum* may be selected during re-seeding efforts on landscapes invaded by *Bromus tectorum*, and identification of selected traits can guide selection and breeding of improved restoration materials.

The goal of this research is to determine if root traits of native perennial grasses lead to successful establishment under *Bromus tectorum* competition and to determine if selection for root traits is occurring during restoration. Two steps are needed: (1) to determine if root traits co-vary with competitive ability in the commercially available *Elymus* germplasms; and (2) determine if field established plants have different root traits compared to the population mean of commercial seed from which they were planted, which would indicate selection during field establishment. A greenhouse mesocosm study was used to address the first question. Root production, tiller production, soil water use, photosynthesis, nutrient acquisition, and competitive ability

was measured and analyzed to investigate whether traits vary among commercially available germplasms and whether the variation is related to competitive ability. We expected that given different evolutionary histories of the germplasm collections, they would vary in their physiology and competitive ability and that competitive ability would be associated with high root production and water acquisition. The second question is whether a consistent selection for root morphological traits occurs for those plants that establish in the field after restoration seedings, and whether such selection is for competitive root traits. Seeds from original commercial seed sources and seed from plants that established in the field after restoration planting in two sites in Elko County were collected and grown in a common garden. Plants from the common garden were planted into small pots in a greenhouse, and root systems of the grasses were sequentially harvested over time to determine variation in root growth rate, production, and morphology using WinRhizo imaging and quantification. Root traits and competitive ability in the commercial and field collected seed were compared to determine if a shift in traits had occurred. We expected to see shifts in traits related to the acquisition of belowground limiting nutrients which are important in competitive interactions.

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Chapter 1. Are competitive tolerance and effect related to physiological differences in nutrient acquisition between *Elymus* spp. and *Bromus tectorum*?

Summary

1. Water and nitrogen are the most limiting resources in arid rangelands. The ability of plants to tolerate abiotic and biotic resource limitations as seedlings and to compete for limiting resources as mature plants determines plant fecundity.
2. Physiological attributes of plant root and shoot systems are related to the ability to acquire nutrients.
3. The goal of this research was to determine if physiological attributes related to nutrient acquisition of *Elymus* spp. (squirreltail) lead to successful tolerance to (competitive response; year 1) and suppression of (competitive effect; year 2) *Bromus tectorum* (cheatgrass) when in competition.
4. We used a greenhouse mesocosm composed of replicate 200 liter barrels filled with soil and instrumented with minirhizotron and neutron probe tubes through the soil profile. *Elymus* plants of five commercially available germplasms were planted in monoculture and in competition with *Bromus tectorum*. Physiological measurements of root production, tiller production, water use, and photosynthesis were taken bi-weekly.
5. *Synthesis*: Competition for water and nitrogen are important for plant performance in the Great Basin and may determine the ability of plants to succeed. We did not detect

large and consistent differences amongst germplasms in their physiology and competitive ability. However, all *Elymus* germplasms were similar in their competitive tolerance to *Bromus tectorum* as seedlings and had a large competitive effect on *Bromus tectorum* as mature plants. The competitive effect of *Elymus* on *Bromus tectorum* production was correlated with water use, but not with root or tiller production. Plant selection for effective restoration of arid rangelands should focus on the ability of plants to induce competitive effect through water acquisition.

Key Words: Great Basin, *Elymus elymoides*, *Bromus tectorum*, competition, competitive effect, competitive response, competitive tolerance, water, physiology, root production.

Introduction

Water and nitrogen are the most limiting resources in arid lands (Smith & Nowak 1990), and spatial and temporal deployment of roots in these systems are a key strategy for successful invasion and dominance by alien species (Harris & Wilson, 1967; Evans *et al.* 1970; Eissenstat & Caldwell 1988). Water is seasonally available in the winter and early spring in the Great Basin (West 1983) and declines with evapotranspiration over the growing season (Campbell & Harris 1977; Dobrowolski, Caldwell & Richards 1990). Nitrogen availability in arid systems depends on pulses of water for microbial

breakdown of organic carbon and enzymatic nitrification (Austin *et al.* 2004), and roots rapidly respond to capture available nitrogen (Cui & Caldwell 1997). Competition occurs early in the growing season between established perennials and seedlings as soil moisture is used (Melgoza, Nowak & Tausch 1990; Reichenberger & Pyke 1990; James *et al.* 2006). Thus, the ability to access moisture and nitrogen early through high root growth rate, allocation to roots, and elongation at low temperatures is important for accessing limiting nutrients (Harris & Wilson 1967; Ross & Harper 1972; Silberbush & Barber 1983).

For restoration to occur in invaded areas of the Great Basin, restoration plant material must be able to compete with *Bromus tectorum* belowground in disturbed settings (Eissenstat & Caldwell 1989; Humphrey & Schupp 2004). The identification of key plant traits related to competitive resource acquisition is an important step in finding plant restoration materials with the highest probability for success in these systems. Analysis of competitive mechanisms in arid and semi-arid systems should focus on root traits due to ecosystem resource limitations and annual grass competition.

Bromus tectorum employs ruderal nutrient acquisition strategies, including a high root relative growth rate (Arrendondo, Jones & Johnson 1998), rapid production of very fine roots, and alteration of root production and morphology in response to changes in soil water conditions (Harris & Wilson 1967; Johnson & Aguirre 1991). *Bromus tectorum* can rapidly exhaust soil water (Evans *et al.* 1970; Cline, Uresk & Rickard

1977), has a high uptake rate for nitrogen and other nutrients (Melgoza, Nowak & Tausch 1990; Lowe, Lauenroth & Burke 2002; Monaco *et al.* 2003,) and effectively competes for limiting nutrients against native perennials (Evans *et al.* 1970; Aguirre & Johnson 1991). *Bromus tectorum* production responds highly to the interaction of water and nitrogen in combination (Link *et al.* 1995). A closely related species (*Bromus madritensis ssp. rubens*) was able to preemptively use soil water resources prior to perennials in the Mojave Desert, which is characterized by similar seasonal precipitation dynamics to the Great Basin (DeFalco, Fernandez & Nowak 2007).

Elymus species (*Elymus elymoides* and *Elymus multisetus*) have been identified as good candidates for restoration of *Bromus tectorum* invaded areas due to their early germination (Young, Clements & Jones 2003), short time to reproduction (often 2 years after sowing), cool season growth, and competitive ability against annual grasses (Jones 1998). Once established, *Elymus* spp. are effective competitors against *Bromus tectorum* (Humphrey & Schupp 2004). *Elymus elymoides* and *E. multisetus* germplasms have been collected from different locations in Idaho and Nevada and then propagated on farms for use in restoration. The collections were evaluated for aboveground production, seed size, and ability to be harvested for commercial releases as restoration material (Jones *et al.* 1998; 2004a; 2004b; Jones 2010). *Elymus* is highly self-pollinating (Jensen, Zhang & Dewey 1990), and thus commercially available *Elymus* germplasms are collections from different locations and represent distinct sets of inbred lines. Because germplasms were

subject to site variations such as soil water availability, nutrient availability, competitive pressure, and different evolutionary trajectories, plant traits related to nutrient acquisition likely vary among germplasms (Ackerly *et al.* 2000; Reich *et al.* 2003). Germplasms of *Elymus* also likely differ in their competitive ability with *Bromus tectorum* due to the effect of regional abiotic variation on competitive traits and different levels of *Bromus tectorum* pressure. However, little is known about the differences amongst germplasms in root characteristics, physiological performance, and competitive ability.

The goal of this study is to measure traits of germplasms of *Elymus* with and without competition with *Bromus tectorum* in order to determine if differences in root systems, aboveground production, water use, and photosynthetic carbon gain exist among the germplasms. We predicted that *Elymus* traits related to the acquisition of water and nitrogen such as high root production rate, allocation to roots, and production of roots at soil layers with high water content would be correlated with nutrient and water acquisition. Efficient acquisition of water and nutrients is a competitive trait in arid lands, and thus we predict that acquisition of nutrients and water by *Elymus* seedlings will be correlated with competitive ability. This experiment investigated the following questions: 1) Do production traits vary among germplasms of *Elymus* and is variation correlated with competitive ability against *Bromus tectorum*?

And 2) Do competitive root traits result in higher nutrient acquisition and improved water acquisition?

Null hypotheses that were tested in this study are:

1. Germplasms of *Elymus* will not differ in their root and shoot production, photosynthesis, or in their ability to acquire water.
2. Germplasms of *Elymus* will not differ in their competitive ability against *Bromus tectorum*.
3. Plant traits and acquisition of water and nitrogen will not be correlated with competitive ability.

Materials and Methods

Seeds of five *Elymus* (*Elymus elymoides* and *Elymus multisetus*) germplasms used in the study were obtained from Tom Jones at USDA-ARS in Logan, Utah and included Sand Hollow (*Elymus multisetus*), Rattlesnake (*Elymus elymoides* ssp. *elymoides*), Toe Jam Creek (*Elymus elymoides* ssp. *californicus*), Fish Creek (*Elymus elymoides* ssp. *elymoides*), T-1735 (*Elymus elymoides* ssp. *californicus*), and Shaniko Plateau (*Elymus elymoides* ssp. "C"). The taxonomic relationships of *Elymus* species and subspecies have been extensively debated and revised (Barkworth, Dewey & Atkins

1983; Dewey 1983). Recent genetic evidence suggests that the sympatric species *E. elymoides* and *E. multisetus* are closely related (Larson *et al.* 2003). Thus, for evaluation of differences in physiology and competitive ability, we feel that inclusion of available restoration materials of both species and subspecies is justified. *Bromus tectorum* seed for the study were from a common population collected near Bordertown, NV.

Elymus seeds were planted with and without *Bromus tectorum* competition in 200 liter barrels in a greenhouse at the University of Nevada, Reno. Barrels were filled with low fertility commercial top soil to 0.70 m depth (soil surface area = 0.24 m², soil volume = 0.166m³). Seeds were planted at a common density of 15 live seeds and thinned to 10 *Elymus* plants per barrel. *Bromus tectorum* was planted in competition and monoculture at a density of 36 live seeds per barrel (200 seeds/m²), which is sufficient to produce competitive effects in native plants. Soil in the barrels was initially watered to maximum drained water holding capacity and the surface was kept moist for germination. After seedlings were established and each of the 10 *Elymus* plants had two fully expanded leaves, no additional water was added to the barrels in order to mimic the natural spring cycle of soil dry down. The first year of the experiment ended when *Bromus tectorum* senesced, at which time aboveground plant biomass was harvested for both species. The barrels were watered at the end of the growing season with sufficient water to allow *Elymus* plants to survive for a second year of measurements. In the winter of year 2, all barrels were again watered to maximum drained water holding

capacity. *Bromus tectorum* was re-sown in the same barrels as the first year at 36 seeds per barrel in the winter of the second year in order to investigate nutrient acquisition and competitive effect of established *Elymus* plants.

Eight replications of each of five germplasms of *Elymus* were planted both in monoculture and in competition with *Bromus tectorum* (8x5x2=80). The experimental design was a randomized complete block design with 2 fixed factors (germplasm and *Bromus tectorum* presence / absence) and a random blocking factor. Blocking was necessary to reduce experimental error due to differences in greenhouse conditions from patterns in air flow and light. Plants within barrels are not independent; therefore barrels are the level of the treatment replication. Eight barrels of *Bromus tectorum* planted in monoculture were used as controls for root production, soil water use, and *Bromus tectorum* biomass production and photosynthesis in the absence of *Elymus*. Eight unplanted barrels were used as controls for barrel water loss in the absence of any plants. Soil volumetric water content, root production, aboveground production, and photosynthesis were measured bi-weekly after seedlings were established. Data was collected at the same time intervals to allow temporal correlation of production with water use and photosynthesis.

Root production was measured using a minirhizotron image capture system (Bartz Technology Corp, Carpintiera, Ca, <http://www.bartztechnology.com>). Minirhizotrons offer a non-destructive method of observing and quantifying production

of individual roots. Recent developments in camera imaging, analysis software, and other hardware have facilitated the collection, analysis, and resolution of minirhizotron data (Hendrick & Pregitzer, 1996; Johnson & Meyer 1998, Johnson *et al.* 2001).

Minirhizotron images were collected weekly at 0.01 m depth increments along the 0.70 m depth of clear plastic tubes inserted at 30 degrees from vertical into the barrels (Fig. 1). Root standing crop over time and by depth was quantified using the computer program RootTracker (David Tremmel, Duke University). Due to spatial variability of root production, root length density per image viewing area (meters of root length per square meter of viewing area) was summed for three depth increments corresponding to soil water measurements (0.20, 0.40, and 0.60 m below the soil surface). Roots in minirhizotron images cannot be reliably differentiated by species; thus root production in competition barrels represents the sum of root length density of both species.

Soil water content was measured using a neutron probe device (CPN 503DR hydroprobe, ICT International, www.ictinternational.com). Neutron probes allow repeated, non-destructive measurement of volumetric soil water content over the soil profile. A 0.80 m aluminum neutron probe access tube was inserted vertically into each of the barrels at 0.10 m from the barrel center and extending to the bottom of the barrel (Fig. 1). Standard counts were taken on each sampling date in order to account for random fluctuations of the probe and decay of the neutron source over time. Although tubes were slightly offset from the center of the barrel due to possible

confounding effects of the plastic from the minirhizotron tube on soil water content measurements, comparisons of neutron probe counts in dry soil with and without minirhizotron tubes did not indicate any significant effect of the minirhizotron tubes on neutron probe count. Neutron probe counts were converted to volumetric soil water content through regression with soil water content from soil cores. Measurements of soil water content were made at 0.20 m, 0.40 m, and 0.60 m below the soil surface.

Photosynthesis was measured mid-day using a Licor 6400 gas exchange system. The conditions used for the measurements were constant at all sampling dates under saturating light conditions ($1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), ambient leaf temperature (20-23°C), extracellular CO_2 concentration of 400 ppm, and intercellular CO_2 concentration of 200-350 ppm. Measurements were conducted on the youngest fully expanded leaf of an *Elymus* plant and *Bromus tectorum* plant (if present) selected at random.

Tillers were counted for all plants in each barrel every two weeks at the same time as other physiological measures. Aboveground biomass of both species was harvested, dried, and weighed at the end of each growing season after *Bromus tectorum* senesced. Nitrogen pool was determined by determining the nitrogen concentration of a composite sample of leaf and stem tissue of the final harvested aboveground biomass and multiplication by total biomass of the barrel. Nitrogen concentration in aboveground tissue was measured by homogenizing the tissue in a ball mill and using a

flash combustion/chromatographic separation technique with a Costech ECS 4010 CHNSO elemental analyzer (www.costechanalytical.com).

Data analysis

Root production, aboveground production (tiller count), water use, and photosynthesis were analyzed using a mixed model ANOVA with the GLIMMIX procedure of SAS 9.3 (SAS institute, Cary, NC, USA www.sas.com). Data was checked for violations of ANOVA including homogeneity of variance among treatment groups, significantly influential outliers, and normal distribution of residuals (Zuur, Leno & Elphick 2010). When necessary to meet the assumptions of ANOVA, the BOXCOX procedure was used to select an appropriate transformation. Adjustment to p values for multiple pairwise comparisons used Tukey's methods.

We first tested null hypothesis 1: that germplasms of *Elymus* will not differ in their above or belowground production, photosynthesis, or in their ability to acquire water. Data for root production and water use was analyzed as a 5 x 2 qualitative factor design with germplasm and *Bromus tectorum* presence/absence as fixed factors and block, depth interval, and time as random factors. Instantaneous photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ sec}^{-1}$) at ambient conditions and saturating light was used as a response variable to test if germplasms differ in their ability to maintain photosynthesis under *Bromus tectorum* competition. Tiller count was used as a response variable to test whether *Elymus* germplasms differ in their ability to produce leaf area for the capture of CO_2 and

for use of water. The design for photosynthesis and tiller production was 5 x 2 fixed interacting factors (Germplasm and *Bromus tectorum* +/-) and two random factors of time and block. For all these ANOVA's, a significant interaction of germplasm and *Bromus tectorum* was examined for differences amongst germplasms in their response to *Bromus tectorum* competition. Further investigation of the least square means and least square differences were used to determine the direction and magnitude of the response.

We tested null hypothesis two, that germplasms of *Elymus* will not differ in competitive ability against *Bromus tectorum* using final biomass data and nitrogen acquisition as response variables. Data was analyzed as a 5 x 2 fixed factor factorial design with the factors germplasm and *Bromus tectorum* presence or absence and a random factor of block to test for competitive tolerance of *Elymus* germplasms to *Bromus tectorum* competition. The interaction of germplasm and *Bromus tectorum* competition on biomass and nitrogen pool of *Elymus* was used to determine whether germplasms differed in competitive tolerance to *Bromus tectorum* competition. Competitive effect of *Elymus* on *Bromus tectorum* was assessed by testing for differences amongst germplasms in competition with *Bromus tectorum* using *Bromus tectorum* production and nitrogen pools as response variables. Data was analyzed as a single fixed factor design with germplasm as the fixed factor and block as a random factor. The significance of the germplasm factor on biomass and nitrogen pool of

Bromus tectorum was used to determine whether germplasms differed in competitive effect on *Bromus tectorum*.

To test null hypothesis 3, that physiological measures of *Elymus* nutrient acquisition will not be correlated with competitive effect, the effect of root production, tiller production, and soil water use on biomass and nitrogen pool of *Bromus tectorum* was determined. We included biomass and nitrogen pool values as quantitative factors in the full ANOVA model of physiological measures described for hypothesis 1 above. The analysis was only done for the competitive effect of *Elymus* on *Bromus tectorum* for year 2 due to the large effect on soil water use and root production by *Bromus tectorum* and the lack of effect of *Elymus* on *Bromus tectorum* in year 1.

Results

In year 1, *Bromus tectorum* had a significant effect on root production in the barrels at all depths after 2 months of growth (*Bromus tectorum* *Time interaction), indicating that root production under competition was dominated by *Bromus tectorum* (Table 1A; Fig. 2A, left panels). Germplasms did not significantly differ in root production in monoculture or in competition with *Bromus tectorum* at any soil depth. Root production was higher and occurred at a faster rate at the 0-0.20 m and 0.20-0.40 m

depths than the 0.40-0.60 m depth (Depth*Time interaction). Soil water use followed the pattern of root production: *Bromus tectorum* increased soil water use after 6 weeks at all depths (*Bromus tectorum* *Depth*Time interaction) (Table 1A; Fig. 3A, left panels). Barrels with *Bromus tectorum* used water much faster than *Elymus* monoculture barrels and dried the soil more completely than *Elymus* was able to do alone. Soil water was used from the 0-0.20 m depth and 0.40 m depth earlier than the 0.40-0.60 m depth (Depth *Time interaction), but total water use was highest from the 0.40-0.60 m depth due to higher amount of water at depth. Germplasms did not significantly vary in their water use, in their response to *Bromus tectorum* presence, or in water use at any depth or over time (Table 1A; Fig. 3A, left panels). Photosynthesis of *Elymus* in year 1 was negatively affected by the presence of *Bromus tectorum*, and the effect was greater at later dates (*Bromus tectorum* *Time interaction) (Table 1A; Fig. 4A, left panel). Photosynthesis of germplasms did not differ in response to *Bromus tectorum* over time (Germplasm **Bromus tectorum* *Time interaction was not significant) (Table 1A; Fig. 4, left panel). The germplasm “T1735” did have significantly higher photosynthesis than “Shaniko”, “Fish Creek”, and “Rattlesnake”, but not significantly higher than “Toe Jam Creek” (Germplasm effect). Tiller production was decreased by the presence of *Bromus tectorum* at all dates (Table 1A; Fig. 5, left panel). Tiller production of the germplasms responded similarly to *Bromus* competition over time (Germplasm**Bromus tectorum* *Time interaction was not significant). The germplasm “Shaniko” produced significantly

more tillers than "T1735", "Toe Jam Creek", and "Fish Creek", but not "Rattlesnake" (Germplasm effect).

In year 2, *Elymus* was more competitive against *Bromus tectorum*, and we found more differences amongst germplasms of established *Elymus* plants than in year 1. Unlike year 1, root production of the germplasms did significantly vary over time: "Fish Creek", "Toe Jam Creek", and "Rattlesnake" all produced higher amounts of roots than "Shaniko" at all dates in the last 58 days of growth, and other pairwise differences amongst germplasms were significant at later dates (Germplasm*Time interaction). *Bromus tectorum* presence had a significantly negative effect on root production at all dates in the last 33 days of growth (*Bromus tectorum**Time interaction) and the effect was more negative at the 0-0.20 m depth than the other depths (*Bromus tectorum* *Depth interaction) (Table 1B; Fig. 2B, right panels). The interaction of *Bromus tectorum* and germplasm factors on root production was not significant at any depth or over time, indicating that the germplasms had similar response to competition by *Bromus tectorum* (Germplasm**Bromus tectorum* *Depth *Time interaction). Similar to root production, *Bromus tectorum* had a significant negative effect on soil water use at all depths, and the effect occurred earlier for deeper depths (*Bromus tectorum* *Depth * Time interaction) (Table 1B; Fig. 3B, right panels). Soil water use of the germplasms was significantly different over time: in the last 66 days of growth, differences amongst germplasms were significant, and in general "Shaniko" had the lowest water use and

“T1735” had the highest. Photosynthesis of *Elymus* germplasms was positively affected by *Bromus tectorum* presence in year 2, especially at later dates (*Bromus tectorum**Time effect) (Table 1B; Fig. 4, right panel). The germplasm “T1735” had the highest photosynthetic rate of the germplasms. *Bromus tectorum* had a continued negative effect on the number of *Elymus* tillers in Year 2 (Table 1B; Fig. 5, right panel). Germplasms responded differently to the presence of *Bromus tectorum*, and “Shaniko” had the smallest decrease in tiller number under *Bromus tectorum* competition compared to monoculture. *Bromus tectorum* had a significant effect later in the growing year (significant *Bromus tectorum**Time interaction).

In year 1, *Elymus* aboveground production was significantly decreased by the presence of *Bromus tectorum*, and the biomass of *Elymus* in competition was less than a tenth of the biomass produced in monoculture (Table 2A; Fig. 6). Germplasms did not significantly vary in their biomass production under *Bromus tectorum* competition or in monoculture (Table 2A). *Bromus tectorum* presence had a significant negative effect on *Elymus* nitrogen pool (*Elymus* in monoculture had 0.40 grams per barrel and *Elymus* in competition had 0.01 grams per barrel) (Table 2A). Nitrogen pool did not significantly vary by germplasm or in the interaction of germplasm and *Bromus tectorum*. *Bromus tectorum* biomass and nitrogen pool was not significantly affected by competition with *Elymus* in year 1 (Table 2A; appendix Fig. 1). Competitive tolerance in year 2 was similar to year 1: germplasms again did not differ in their competitive tolerance to *Bromus*

tectorum competition (Germplasm**Bromus tectorum* effect not significant). The germplasm effect was also not significant in year 2 (Table 2B; appendix Fig 2). However, unlike year 1, *Elymus* germplasms had a large competitive effect on *Bromus tectorum* biomass production (Table 2B, Fig. 7). Germplasms also differed in competitive effect on *Bromus tectorum* in both biomass and nitrogen acquisition: “T1735” had a significantly lower effect than the other germplasms.

Root production and *Elymus* tiller production did not have significant effect on *Bromus* biomass (root production: $f=1.97$, $p=0.160$; tiller production: $f=0.61$, $p=0.436$). Soil water use did have a significant effect on the interaction of germplasm and biomass ($f=2.84$, $p=0.024$), indicating that the competitive effect of *Elymus* on *Bromus tectorum* was related to soil water use and that the effect varied by germplasm. Further investigation of the interaction indicated that the germplasm “Shaniko” had higher competitive effect than other germplasms on *Bromus tectorum* through soil water use when *Bromus tectorum* biomass was relatively low. The effect of root production, tiller production, and soil water use on nitrogen pool were not significant (root production: $f=0.24$, $p=0.622$; tiller production: $f=0.30$, $p=0.583$; soil water use: $f=0.56$, $p=0.452$).

Discussion

Elymus germplasms had few differences in their physiology related to nutrient acquisition (hypothesis 1) or competitive ability (hypothesis 2). The germplasms showed little difference in either year in root production or soil water use, except for higher amounts of water use in the germplasm “T1735” during the last months of growth in year 2. *Bromus tectorum* had similar suppressive effect on the ability of germplasms to produce roots and access water in year 1, indicating that the physiological response to competition did not differ by germplasm. However, the direction of *Bromus tectorum* effect on root production and soil water use switched in the 2 years: in Year 1 *Bromus tectorum* had a positive effect, and in year 2 *Bromus tectorum* had a negative effect. In year 2, *Elymus* monoculture barrels had higher root production and water use than competition barrels despite the presence of *Bromus tectorum*, most likely due to competitive effect of *Bromus tectorum* on *Elymus* in year 1 combined with the poor performance of *Bromus tectorum* in competition in year 2.

Bromus tectorum biomass, nitrogen pool, root production, and water use was much less in year 2 than in year 1. A few things might be responsible for the differences. First, a flush of nutrients may have been readily available in the soils during the first year due to the assart effect from soil disturbance and removal of plants (Kimmins, 2004). Second, biomass was removed after year 1 in the competition barrels, and nutrients from the removed biomass was not replaced. Soils were initially low in nutrients, and

the removed amount of nutrients may have negatively affected the production in year 2.

Photosynthesis of the germplasms had slight differences in both years (germplasm “T1735” had significantly higher photosynthetic rate), but germplasms did not differ in their ability to maintain photosynthesis under *Bromus* competition. The photosynthetic differences amongst germplasms were consistent between years despite differences in water availability and root production. However, the direction of the *Bromus tectorum* effect switched between years. In year 1, photosynthesis of *Elymus* was negatively affected by *Bromus tectorum*. Photosynthesis of *Elymus* germplasms was positively affected by *Bromus tectorum* presence in year 2, especially at later dates (*Bromus tectorum* *Time effect) (Table 1B; Fig 4, right panel). This effect was probably due to reduced *Elymus* leaf area in the competition barrels and poor growth of *Bromus tectorum* in year 2 acting to reduce water use and allow *Elymus* germplasms to maintain high photosynthesis rates. Photosynthesis of *Bromus tectorum* was not significantly affected by the presence of *Elymus* in either year (Year 1: $f_{5, 108}=1.91$, $P=0.099$; Year 2: $f_{5, 240}=1.88$, $P=0.099$) (appendix Fig 3). The lack of differences in photosynthesis of *Bromus tectorum* with and without competition with *Elymus* does not help to explain the competitive effect of *Elymus* on *Bromus tectorum* in Year 2.

Competitive tolerance of *Elymus* seedlings did not differ by germplasm, but as mature plants, *Elymus* germplasms did differ in their competitive effect on *Bromus*

tectorum. The germplasm “T1735” had a slightly lower competitive effect than the other germplasms in year 2. *Elymus* seedlings in year 1 in competition were very small, and were significantly affected by *Bromus tectorum*. After establishment, *Elymus* plants had a large effect on *Bromus tectorum* production and nitrogen acquisition. These results suggest that establishment of *Elymus* plants is a key step in restoration and that mature *Elymus* plants have a significant competitive effect on *Bromus tectorum*.

We found one correlation between physiological measures of nutrient capture and competitive effect of *Elymus* on *Bromus* by established *Elymus* plants. The competitive effect of *Elymus* was related to soil water use: soil water use had a negative relationship to *Bromus tectorum* biomass production under competition. The relationship of root production to competitive effect of *Elymus* was not significant, but the effect did have the same negative direction as soil water use. Thus although the direct effect of root production on *Bromus tectorum* production was not strong, the relationship of root production on competitive effect through acquisition of water is supported. Results from hypothesis 3 are consistent with the importance of competition for water in the suppression of *Bromus tectorum*.

We expected that due to differences in biotic and abiotic site conditions from which they were collected, plant traits were likely to vary among germplasms. Seed collection sites were geographically separated, varied in elevation (830 m to 1829 m), and varied in mean annual precipitation (280 to 355 mm averages) (Jones *et al.* 1998;

2004a; 2004b; Jones 2010). Seed from germplasm source plants were collected from relatively intact native communities, but differed in the composition of plants at the collection sites. We do not know the variation in the intensity or duration of *Bromus tectorum* competition at the sites in which the germplasms were collected, but at least 2 of the collections (Rattlesnake and Fish creek) were growing in the presence of *Bromus tectorum* (Jones *et al.* 2004b, 2010). None of the collections were from remnant plants in heavily invaded *Bromus tectorum* fields, which have been shown to cause selection of plants through differential survival (Leger 2008). Lack of variation in physiology and response to *Bromus tectorum* may be due to insufficient time or stress for differences to develop, or a high degree of phenotypic plasticity in this species which may preclude local adaptation (Sultan 1987).

Our results are consistent with differences in the importance of competitive tolerance and effect of perennial plants at different life stages. Although both competitive tolerance and effect are important throughout the life of a plant, for *Elymus* restoration, competitive tolerance is more important at the seedling establishment stage and competitive effect is more important in mature stages. As seedlings, *Elymus* and *Bromus tectorum* compete for shallow soil resources, and *Elymus* establishment depends on the tolerance of seedlings to rapidly drying soil as water is used by *Bromus tectorum*. The presence of *Bromus tectorum* had a large effect on root zone exploitation of soil water and nitrogen availability to *Elymus* seedlings in this study. *Elymus* seedling

photosynthesis, aboveground production, and nitrogen acquisition was negatively affected by the presence of *Bromus tectorum*, but *Elymus* had no significant competitive effect on *Bromus tectorum* production in year 1 (Table 2A; appendix Fig. 1). *Bromus tectorum* had a very rapid rate of root production and soil water use when not in competition with established *Elymus* plants in year 1. As established plants, *Elymus* was able to rapidly access soil water resources from both shallow and deep depths and limit *Bromus tectorum* growth and nitrogen acquisition.

Establishment of native plants has proven difficult and is a key step in restoration of *Bromus tectorum* invaded rangelands. Selecting plants that have good competitive tolerance and effect against *Bromus tectorum* is a goal of plant breeding and selection. Similarities in physiology of *Elymus* seedlings in monoculture barrels in year 1 suggest that there might not be a lot of differences amongst the currently available germplasms if establishment is dependent on physiology. Correlations of physiology and competitive ability of all plants regardless of germplasm (hypothesis 3) suggest that soil water use is important in competitive relationships. Competitive effect and tolerance are often correlated (Goldberg & Landa 1991), thus measuring the ability of restoration collections to effectively compete for water may be an important part of plant breeding and selection.

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Tables

Table 1. ANOVA results for physiological response variables in years 1 (2011, A) and 2 (2012, B). Response variables are presented in each column. Numerator degrees of freedom, denominator degrees of freedom, F values and P values are listed in the table for each effect and interaction under each response variable. Significant effects ($p < 0.05$) are highlighted in bold in the table. Results were generated using the GLIMMIX procedure in SAS 9.3.

A. Year 1 Physiological measures		Root production		Water use		Photosynthesis		Tillers	
Effect	(NDF, DDF)	(F, P)	(NDF, DDF)	(F, P)	(NDF, DDF)	(F, P)	(NDF, DDF)	(F, P)	
Germplasm	4,70	0.88,0.480	4,70	0.49,0.743	4,70	4.92, 0.002	4,70	4.27, 0.004	
Bromus	1,70	154.7, <0.001	1,70	248.5, <0.001	1,70	140.6, <0.001	1,70	229.16, <0.001	
Germ * Brom	4,70	0.62, 0.651	4,70	0.12, 0.976	4,70	1.29, 0.281	4,70	1.44, 0.229	
Depth	2,140	4.41, 0.014	2,140	48.1, <0.001	-	-	-	-	
Germ*Dep	8,1260	0.55, 0.818	8,1050	1.99, 0.441	-	-	-	-	
Brom*Dep	2,1260	3.63, 0.027	2,1050	49.1, <0.001	-	-	-	-	
Germ* Brom*Dep	8,1260	0.48, 0.869	8,1050	0.43, 0.904	-	-	-	-	
Time	6,1260	421.3, <0.001	5,1050	3130.2, <0.001	3,185	18.95, <0.001	4,280	453.2, <0.001	
Germ*Tim	24,1260	1.05, 0.392	20,1050	0.84, 0.668	12,185	0.85, 0.598	16,280	1.65, 0.056	
Brom*Tim	6,1260	220.3, <0.001	5,1050	453.6, <0.001	3,185	4.33, 0.006	4,280	327.37, <0.001	
Germ* Brom*Tim	24,1260	0.78, 0.765	20,1050	1.44, 0.097	12,185	0.58, 0.857	16,280	1.00, 0.457	
Dep*Tim	12,1260	5.39, <0.001	10,1050	112.6, <0.001	-	-	-	-	
Germ*Dep*Tim	48,1260	0.45, 0.999	40,1050	1.10, 0.304	-	-	-	-	
Brom*Dep*Tim	12,1260	3.73, <0.001	10,1050	59.0, <0.001	-	-	-	-	
Germ* Brom*Dep*Tim	48,1260	0.44, 0.999	40,1050	0.64, 0.962	-	-	-	-	

B. Year 2 Physiological measures		Root production		Water use		Photosynthesis		Tillers	
Effect		(NDF, DDF)	(F, P)	(NDF, DDF)	(F, P)	(NDF, DDF)	(F, P)	(NDF, DDF)	(F, P)
Germplasm		4, 70	2.31, 0.067	4, 70	2.25, 0.072	4, 70	10.33, <0.001	4, 70	10.55, <0.001
Bromus		1, 70	12.79, 0.001	1, 70	181.7, <0.001	1, 70	10.35, 0.002	1, 70	591.8, <0.001
Germ * Brom		4, 70	0.44, 0.777	4, 70	0.72, 0.582	4, 70	0.59, 0.6726	4, 70	9.24, <0.001
Depth		2, 140	8.59, <0.001	2, 140	221.5, <0.001	-	-	-	-
Germ*Dep		8, 2310	1.03, 0.411	8, 1890	1.60, 0.120	-	-	-	-
Brom *Dep		2, 2310	3.95, 0.019	2, 1890	49.3, <0.001	-	-	-	-
Germ* Brom *Dep		8, 2310	0.93, 0.4915	8, 1890	0.52, 0.844	-	-	-	-
Time		11, 2310	547.0, <0.001	9, 1890	3094.3, <0.001	9, 630	26.54, <0.001	11, 770	1048.7, <0.001
Germ*Tim		44, 2310	4.14, <0.001	36, 1890	2.79, <0.001	36, 630	1.58, 0.018	44, 770	3.08, <0.001
Brom *Tim		11, 2310	7.59, <0.001	9, 1890	165.8, <0.001	9, 630	4.91, <0.001	11, 770	224.30, <0.001
Germ* Brom *Tim		44, 2310	0.52, 0.997	36, 1890	0.80, <0.795	36, 630	1.11, 0.309	44, 770	4.60, <0.001
Dep*Tim		22, 2310	19.88, <0.001	18, 1890	316.0, <0.001	-	-	-	-
Germ* Dep*Tim		88, 2310	1.19, 0.117	72, 1890	0.78, <0.907	-	-	-	-
Brom * Dep*Tim		22, 2310	0.96, 0.518	18, 1890	289.0, <0.001	-	-	-	-
Germ * Brom *Dep*Tim		88, 2310	0.88, 0.783	72, 1890	0.39, >0.999	-	-	-	-

Table 2. Competitive tolerance and effect of *Elymus* germplasms grown in competition with *Bromus tectorum*. In year 1 *Bromus tectorum* had a significant effect on *Elymus* production and nitrogen acquisition but germplasms were not significantly different in competitive tolerance. In year 2, *Elymus* germplasms differed in their effect on *Bromus*: the germplasm "T1735" had a significantly lower effect on *Bromus* aboveground biomass production and nitrogen acquisition.

A. Year 1- Competitive tolerance		Biomass- <i>Elymus</i>	Nitrogen pool- <i>Elymus</i>
Effect	(NDF, DDF)	(F, P)	(F, P)
Germplasm	4,63	1.27, 0.290	0.66, 0.619
<i>Bromus tectorum</i>	1,63	264.6, <0.001	233.52, <0.001
Germplasm*<i>Bromus tectorum</i>	4,63	0.99, 0.421	0.64, 0.6339
Year 1- Competitive effect		Biomass- <i>Bromus</i>	Nitrogen pool- <i>Bromus</i>
Germplasm	4,28	1.26, 0.307	0.54, 0.707

B. Year 2- Competitive tolerance		Biomass- <i>Elymus</i>	Nitrogen pool- <i>Elymus</i>
Effect	(NDF, DDF)	(F, P)	(F, P)
Germplasm	4,63	0.43, 0.785	1.58, 0.191
<i>Bromus tectorum</i>	1,63	145.2, <0.001	17.81, <0.001
Germplasm*<i>Bromus tectorum</i>	4,63	1.93, 0.117	2.19, 0.080
Year 2- Competitive effect		Biomass- <i>Bromus</i>	Nitrogen pool- <i>Bromus</i>
Germplasm	4,28	10.45, <0.001	5.71, 0.002

Figures

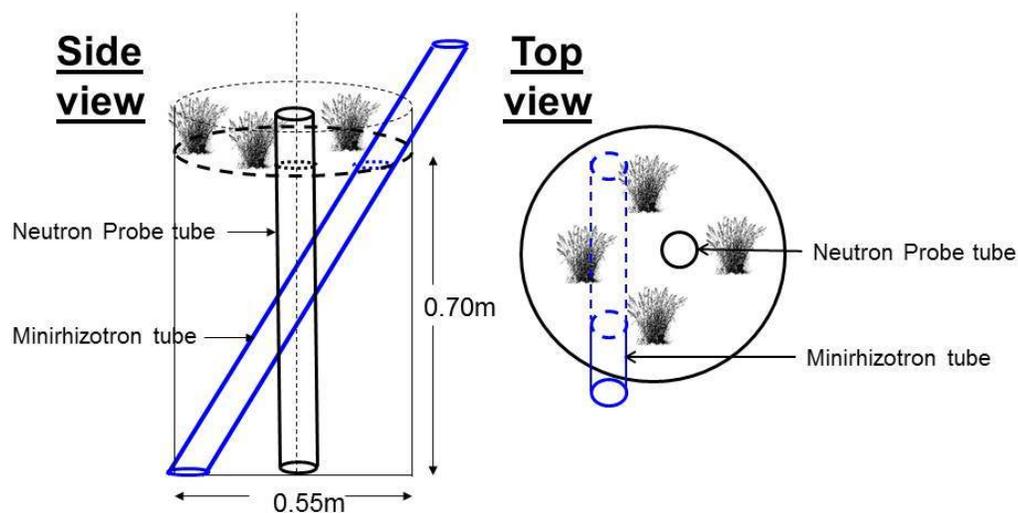


Figure 1. Diagram of the experimental setup for measurement of root production, soil water use, and photosynthesis of *Elymus* germplasms with and without competition with *Bromus tectorum*. A neutron probe access tube is inserted vertically into the barrel 0.10 m from the center of the barrel. A minirhizotron tube is inserted at a 30 degree angle off set from the center of the barrel by 0.10 m in the opposite direction as the neutron probe tube. Both tubes extend to the bottom of the barrel at 0.70 m soil depth. Minirhizotron and neutron probe tubes allow repeated, non-destructive measurements of root production and soil water content over the growing season.

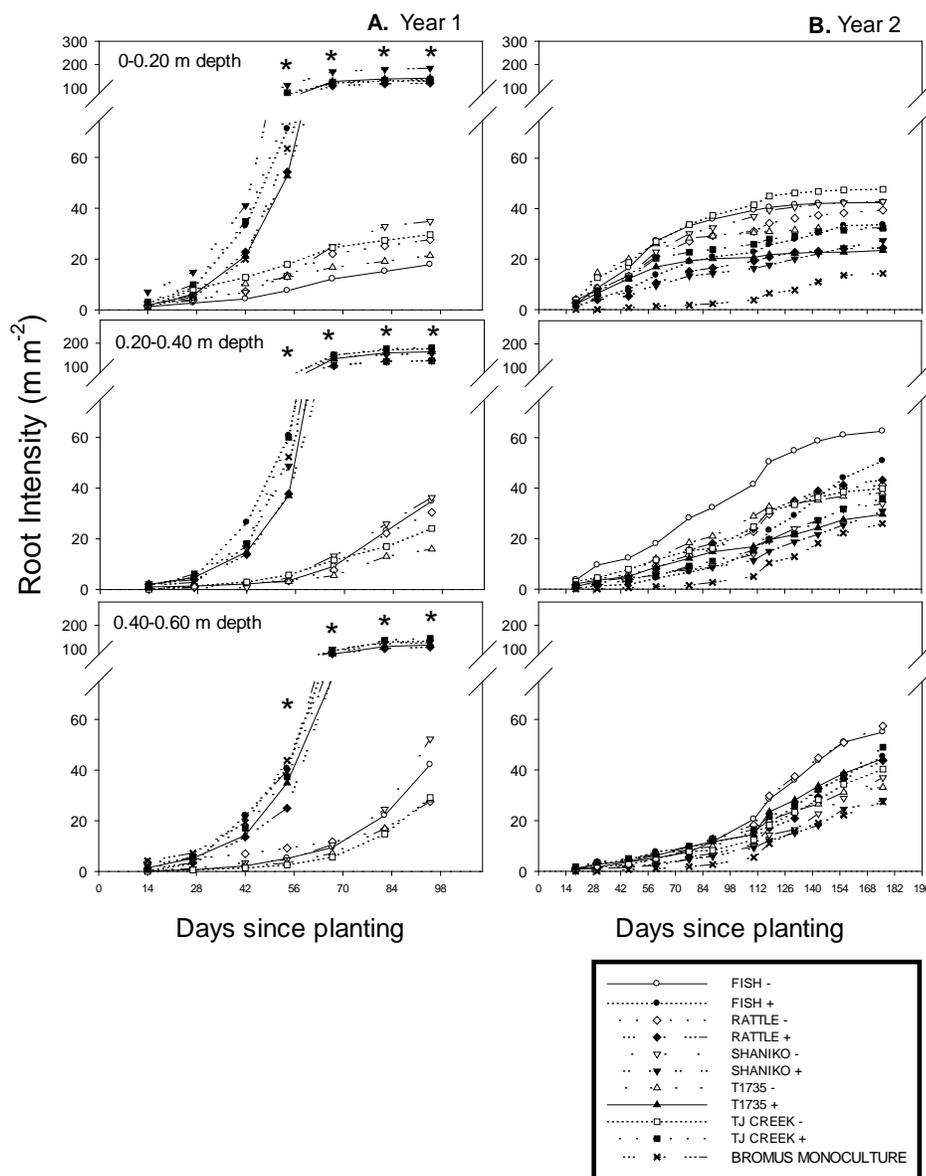


Figure 2. Root length density (meters of root per square meter of image area) of *Elymus* germplasms grown with (dark symbols, “GERMPLASM” +) and without (open symbols, “GERMPLASM” -) competition with *Bromus tectorum*. *Bromus tectorum* monoculture is shown for reference (x symbol), but data was not included in the ANOVA model. Left panel (A) are year 1 in which *Elymus* were seedlings, and right panel (B) are year 2 in which *Elymus* were mature plants. Asterisks indicate a significant effect of *Bromus tectorum* on root production at the indicated time and depth. In year 1, presence of *Bromus tectorum* had a significant positive effect on root production in the barrels. In Year 2, *Bromus tectorum* had a significant negative effect on root production in the barrels.

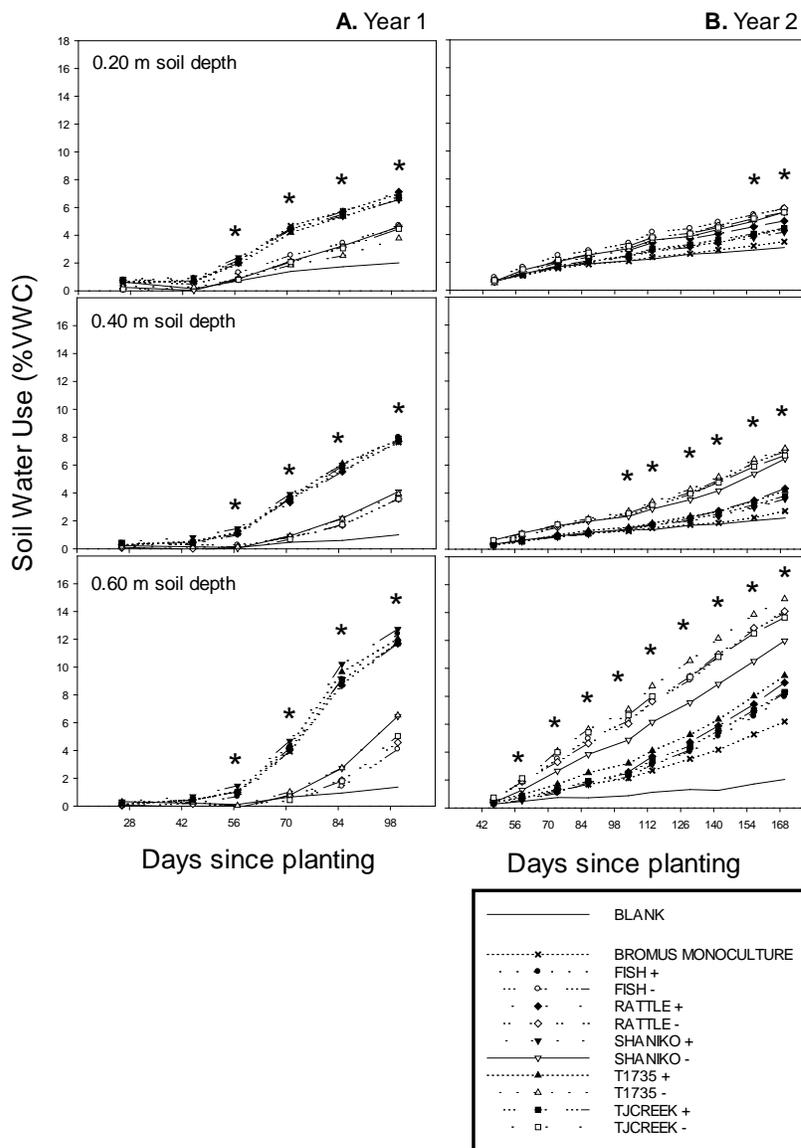


Figure 3. Soil water use (percentage of volumetric water content) of *Elymus* germplasms grown with (dark symbols, “GERMPLASM” +) and without (open symbols, “GERMPLASM” -) competition with *Bromus tectorum*. Left panel (A) are year 1 in which *Elymus* were seedlings, and the right panel (B) are year 2 in which *Elymus* were mature plants. The blank line represents water use from barrels without any plants. *Bromus tectorum* monoculture is shown for reference (x symbol), but data was not included in the ANOVA model. Asterisks indicate a significant effect of *Bromus tectorum* on soil water use at the indicated time and depth. In year 1, presence of *Bromus tectorum* increased water use from the barrels. In Year 2, *Bromus tectorum* decreased water use in the barrels.

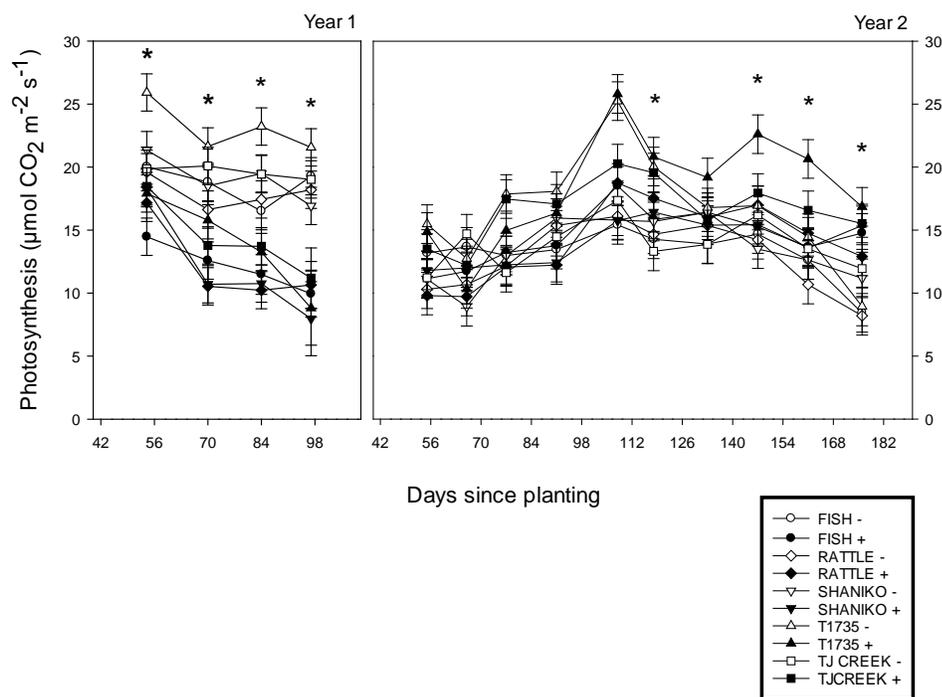


Figure 4. Photosynthesis of *Elymus* germplasms in monoculture (open symbols, “GERMPLASM”-) and competition with *Bromus tectorum* (dark symbols, “GERMPLASM +”). Crosshash symbols indicate a significant *Bromus tectorum* effect at on *Elymus* photosynthesis on the indicated date. *Bromus tectorum* had a significant negative effect on *Elymus* photosynthesis during establishment of the grasses in year 1. *Bromus tectorum* had a significantly positive effect on *Elymus* photosynthesis in year 2. The germplasm “T1735” had an overall higher photosynthetic rate than the other germplasms in both years, but the interaction of germplasm and *Bromus tectorum* was not significant in either year.

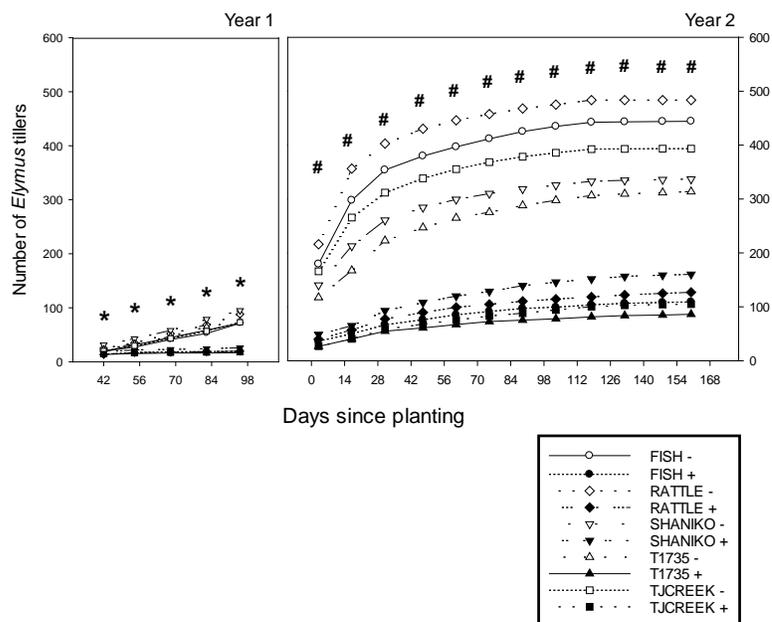


Figure 5. Number of tillers of *Elymus* germplasm produced in monoculture (open symbols, “GERMPLASM”-) and competition with *Bromus tectorum* (closed symbols, “GERMPLASM”+). In both years *Bromus tectorum* competition had a significant negative effect on tiller production of *Elymus*. Asterisks in year 1 indicate a significant *Bromus tectorum* effect on the indicated day. The pound sign symbols in year 2 indicate a significant Germplasm * *Bromus tectorum* interaction on the indicated day.

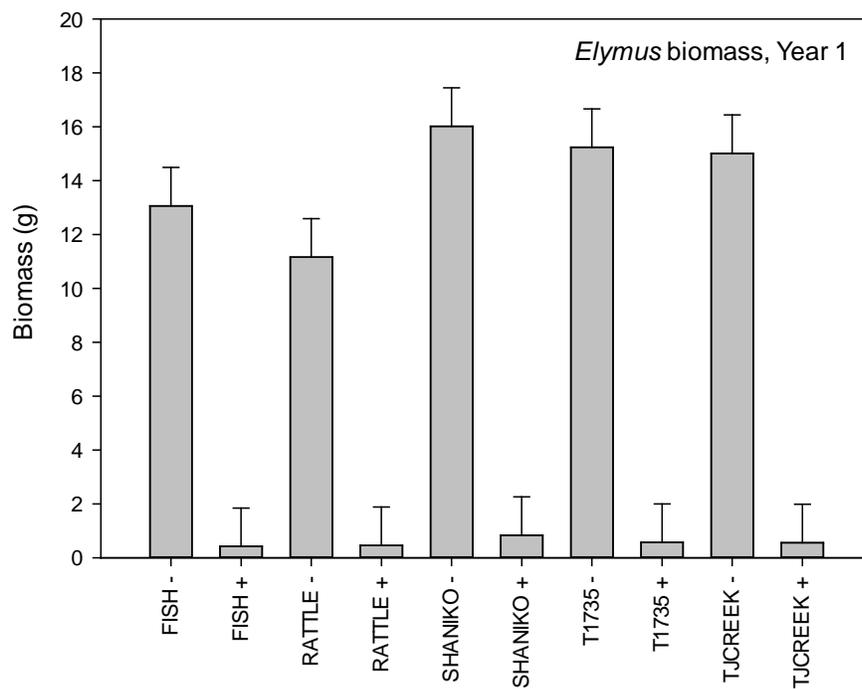


Figure 6. Biomass of *Elymus* germplasms in monoculture (“GERMPLASM” -) and competition with *Bromus tectorum* (“GERMPLASM” +) in year 1 of the experiment. Germplasms responded similarly to *Bromus tectorum* competition (no significant Germplasm * *Bromus tectorum* interaction). *Bromus tectorum* had a significant negative effect on biomass of all *Elymus* germplasms.

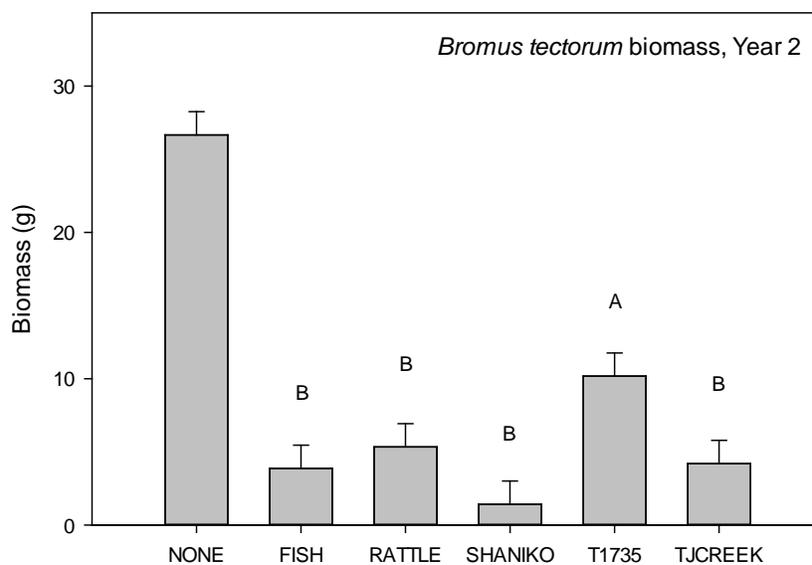


Figure 7. Biomass of *Bromus tectorum* in monoculture and competition with germplasms of *Elymus* in year 2 of the experiment. *Elymus* had a significant competitive effect on *Bromus tectorum* overall, and the germplasm "T1735" had significantly less effect than the other germplasms.

Chapter 2. Evidence for directional selection in traits of *Elymus elymoides* in competition with *Bromus tectorum* in the field.

Summary

1. Introduced plant species can influence ecological processes and cause mortality of native plants.
2. Suites of plant traits are important in competitive interactions. Competitive interactions may result in evolutionary change if the interaction results in natural selection for specific plant traits.
3. *Bromus tectorum* is a common annual weedy grass in the Great Basin and can prevent re-establishment of native grasses in disturbed areas. *Bromus tectorum* is an effective competitor for limiting resources such as water and nitrogen and can pre-emptively access resources through rapid root production and dominance of the shallow root zone.
4. *Elymus elymoides* is a common native bunchgrass in the Great Basin and has been identified as a promising candidate for restoration. Seed of the “Toe Jam Creek” germplasm of *Elymus elymoides* ssp. *californicus* was planted into the field with high levels of *Bromus tectorum* competition in two restoration sites in Nevada. Seed was collected from plants that established in the restoration sites

and evaluated for shifts in plant traits with a focus on root traits related to nutrient acquisition.

5. Results indicate significant directional selection for smaller plants with higher amounts of investment in root biomass. Shifts in root traits were not consistently related to acquisition of soil resources and competitive ability, as measured by the interaction of seed source and *Bromus tectorum* competition on above and belowground biomass.
6. *Synthesis:* This study shows that directional selection for plant traits occurred in two sites in the Great Basin after seeding of restoration plants. The selection was for smaller plants with higher root fraction of the total plant and lower fraction of very fine roots. The results are consistent with selection for traits that allow plants to survive as seedlings in areas of high *Bromus tectorum* cover, but not consistent with selection for nutrient acquisition or competitive ability with *Bromus tectorum*.

Key words: *Elymus elymoides*, *Bromus tectorum*, competition, genetic shift, fine roots, specific root length, directional selection.

Introduction

Species interactions are important in evolutionary processes at multiple time scales (Thompson 1998, 1999). Introduced plant species can influence ecological processes and cause mortality of natives. In response to the survival pressure placed on native communities by invaders, natives have a variety of rapid, evolutionary changes in response to the selection pressure imposed by the invader (Mooney & Cleland 2000; Kinnison & Hairston 2007). For directional selection and evolution in native populations to occur, the fitness of the native population must be affected by the invasive, and traits involved in the fitness of the native must have heritable variation (Strauss, Lau & Carroll 2006). The populations must also be genetically isolated from uninvaded populations so gene flow between the populations does not overwhelm the selection of fitness components (Holt & Gomulkiewicz 1997). Few studies have investigated evolutionary response under changes of plant-plant competitive interactions (Callaway *et al.* 2005; Lau 2006; Meador & Hild 2006; Leger 2008). Selected, competitive traits have rarely been identified in response to species introductions, but see Rowe & Leger (2011).

Suites of plant traits are important in competitive interactions. In a comparative study of wetland plants, competitive ability was predicted from plant traits associated with capture of light including plant size, canopy size, and leaf shape (Gaudet & Keddy 1988). A suite of traits related to acquiring limiting nutrients may be important in native Great Basin grasses, and the traits are likely related to the acquisition of water and

nitrogen rather than light (Casper & Jackson 1997). Over much of the Great Basin and in target restoration areas, *Bromus tectorum* comprises a significant amount of the total plant cover, and *Elymus elymoides* seedlings experience a high level of competition against *Bromus tectorum* for water and nutrients. Nutrient acquisition is important for seedling establishment, and seeds that are able to establish likely have root traits that allow nutrient acquisition under competition. Small diameter roots function primarily in uptake of water and nutrients (Eissenstat *et al.* 2000). Root traits correlated with access to limiting nutrients in arid systems include high relative growth rates, production of small diameter roots (Eissenstat 1992), and high root to shoot ratio (Chapin 1980; Dobrowolski, Caldwell & Richards 1990).

Traits that allow *Elymus* plants to compete with *Bromus tectorum* at the seedling stage may have been selected during re-seeding efforts on landscapes invaded by *Bromus tectorum*. In most cases, a small fraction of re-seeded native grasses establish in fields with high *Bromus tectorum* cover (James & Svejcar, 2010). Although total plant establishment of seeded grasses is low, some plants from field restoration efforts establish and produce seed (Kulpa *et al.*, 2012), and preliminary data indicate a shift toward smaller plants in those plants that are able to establish (Kulpa, 2010). Small aboveground plant size may be a result of a shift in allocation to root production, or simply an overall decrease in plant size without changes to allocation. Small plant size may have been selected because under competition, lower amounts of leaf area can be

supported with decreased available water and nutrients (Hendrix *et al.* 1991; Hendrix & Trapp 1992; Blum & Sullivan 1997). Investigation of shifts in root traits may provide evidence about the mechanisms of successful seedling establishment when in competition with *Bromus tectorum* and indicate which traits would be beneficial for plant breeders to focus their efforts.

Re-seeding efforts use a number of different species including varieties of the perennial bunchgrass Squirreltail (*Elymus elymoides*) originally collected from populations in Idaho and Nevada (Jones 1998, 2004). Commercially available plant seed is selected for large plant size, ease of harvest, and to represent different ecotypes. This research focuses on *Elymus elymoides* because it has a wide natural geographic range and has been identified as a good candidate for restoration (Jones, 1998). *Elymus elymoides* plants are highly self-pollinated (Jensen, Zhang & Dewey 1990) and thus germplasms of *Elymus elymoides* seed likely are a set of inbred lines. *Elymus elymoides* has some morphological characteristics in common with *Bromus tectorum* such as a high specific root length and specific leaf area (Arredondo, Jones & Johnson 1998) and is capable of fall germination cool and season growth (Hironaka & Sinelar 1973).

A pot study was used to determine if root traits and competitive ability differed between commercially available seed and seed from plants that successfully established in the field. The approach was to first compare root traits of plants grown from commercial seeds and seeds from plants that were able to establish in the field in order

to determine if field established plants have a shift in root traits, and second to determine if a shift in root traits is consistent with competitive ability. This research addresses two important questions: 1) Does competition with *Bromus tectorum* in the field during seed establishment cause selection for *Elymus elymoides* plants with specific traits? and 2) Is selection acting on competitive root traits? The null hypotheses to be tested in this study are:

1. Commercially available seed and seed collected in the field after a restoration seeding do not differ in their root traits.
2. Commercially available seed and seed collected in the field after a restoration seeding do not differ in their competitive ability.

Materials and Methods

Elymus elymoides seed of the “Toe Jam Creek” germplasm (*Elymus elymoides* ssp. *californicus*) was obtained from commercially available seed sources and seeded by the U.S. Forest Service into post-fire restoration experimental sites in Elko County, Nevada. After 2 years, seed from established plants was collected from two sites (Humboldt (40°45'36.8"N, 115°50'22.4"W) and Gopher (41°12'4.3"N, 115°20'59.6"W)) and was planted along with the original commercially available seed in a common garden at the Valley Road Field Laboratory near the University of Nevada, Reno (Kulpa,

2010). Common garden planting was used to minimize any possible maternal effects caused by differences in conditions between the restoration field and farm. Because the seeds were from a common garden, differences in traits examined are likely a result of genetic differences and not environmental effects. Seed was collected from individual plants (hereafter referred to as “families”) of each field collection and original commercial seed from the common garden. Seed from the common garden was used in the greenhouse study described below.

Small pots (approximately 600 ml) were filled with low fertility, un-amended Nevada topsoil (Moana Nursery, Reno, Nevada) and watered to settle the soil. Fifteen families of each of the field collections and original seed were picked at random from the available families, and checked to make sure that they represent a normal distribution of plant sizes based on previously collected data. Five replicate plantings from each of the 15 plant families of each of the field collected germplasms and the farmed seed were grown in individual pots with and without *Bromus tectorum* competition. Four seeds of each *Elymus elymoides* family collected from the common garden were sown into individual pots with and without *Bromus tectorum* and randomly thinned to a single individual shortly after germination. *Bromus tectorum* was also sown at a density of 4 seeds per pot and randomly thinned to a single plant per pot. Forty *Bromus tectorum* monoculture pots were planted in order to have a control from which to evaluate the effect of *Elymus elymoides* on *Bromus tectorum* production. Pots were

harvested at four time steps of 30 days each. The total pot number was 15 families x 3 collections x 2 *Bromus tectorum* competition treatments x 4 time steps x 5 replications + 40 *Bromus tectorum* only pots = 1840. Pots were watered every other day, and the greenhouse temperatures were set to 5.0 °C at night and 15.0 °C during the day.

Every 30 days over the experiment, whole plants were harvested, divided into above and belowground fractions, and the root systems were washed from soil. Root system traits (total root length, mean root diameter, and fine root length in diameter classes 0.05-0.10 mm and 0.10-0.15 mm) were determined using a high resolution scanner and the computer program WinRhizo (Regent Instruments Inc, www.regent.qc.ca). WinRhizo analyzes images of root systems and yields data about root system morphology, topology, and architecture. Roots in diameter classes 0.05-0.10 mm and 0.10-0.15 mm were analyzed in addition to total root length because very fine roots are more important for nutrient acquisition than roots of larger diameter. After imaging, root and shoot biomass were dried and weighed. Root fraction of entire plant and specific root length were calculated using measurements of root system mass, shoot system mass, and root length.

Root morphology measures and derived values for each species in each pot were analyzed with analysis of variance (ANOVA) using the GLIMMIX procedure in SAS 9.3 (SAS Institute, Inc., Cary, NC, USA, www.sas.com) with a 0.05 level of significance. The BoxCox procedure in SAS 9.3 was used to make appropriate transformations of the data

in order to meet the normal distribution and homogeneity of variance assumptions of ANOVA, and many of the response variables needed to be transformed prior to analysis. F and p values in the table represent significance measures for the transformed data. The experimental design is a completely randomized design with two fixed interacting qualitative factors of seed sources (Humboldt, Gopher, Commercial) and *Bromus tectorum* presence / absence. Plant family was a nested effect within seed source, and harvest timestep was a random effect. Differences in root traits between seed sources were determined using ANOVA for each response variable. Null hypothesis 1, that commercially available seed and seed collected in the field after a restoration seeding do not differ in their root traits, was tested using root morphology and biomass data. Null hypothesis 2, that commercially available seed and seed collected in the field after a restoration seeding do not differ in their competitive ability, was tested by measuring the competitive tolerance of the *Elymus elymoides* seedlings to *Bromus tectorum* competition. The competitive tolerance was determined for aboveground and belowground production in order to account for any changes to allocation under competition. The tolerance of *Elymus elymoides* to *Bromus tectorum* competition was evaluated as the difference amongst seed sources in *Elymus elymoides* biomass production in monoculture and in competition (Seed source**Bromus tectorum* interaction). Significance of the interaction of Seed source and *Bromus tectorum* was interpreted as seed sources responding differently to the presence of *Bromus tectorum*.

Further investigation of the least square means and least square differences were used to determine the direction and magnitude of the response.

Results

Plants from commercial seed had higher total root length and length of fine and very fine roots (Table1, Fig 2). The interaction of Seed source* *Bromus tectorum* competition* Time was not significant for total root length or fine roots, but it was significant for very fine roots (Fig 2). At 60, 90, and 120 days of growth, very fine root production of commercial seed was less negatively affected by *Bromus tectorum* competition than field selected seed. Average diameter of all roots in the root system was not significantly different amongst seed sources, and the interaction of *Bromus tectorum* *Time step was also not significantly different. Because average diameter was not different amongst seed sources but length was significantly higher in commercial seed than selected seed, root system surface area was significantly higher in commercial seed. The interaction of *Bromus tectorum* competition and Seed source was also significant for root system surface area: commercial seed had a lower decrease in root system surface area between *Bromus tectorum* absence and presence than field collected seed. Both root weight and shoot weight were higher in commercial seed than in field selected seed (Table 1, Fig 3). As a fraction of total plant biomass, field selected seed produced higher root system fraction of total plant mass (Fig 3). Plants from

commercial seed produced a higher fraction of fine roots less than 0.1 mm diameter than field selected seed (fine root fraction of total length, Table 1).

Null hypothesis 2, that commercially available seed and seed collected in the field after a restoration seeding do not differ in their competitive response to *Bromus tectorum* competition was evaluated by looking at the interaction of seed source and *Bromus tectorum* factor in the ANOVA model. Both root weight and shoot weight did not have a significant interaction between seed source and *Bromus tectorum* competition (Table 1, Fig 3) meaning that overall there was no difference in competitive response to *Bromus tectorum* competition amongst seed sources. Length of very fine roots had a significant Seed source**Bromus tectorum** Time step interaction: commercial seed source had a smaller relative decrease in very fine root length between monoculture and competition pots at 60, 90, and 120 days of plant growth (Table 1, Fig 2). Root system surface area had significant Seed source **Bromus tectorum* competition interaction, and commercial seed had a lower relative decrease in root system surface area under *Bromus tectorum* competition. The lower relative decrease in root surface area is consistent with the interaction of seed source and *Bromus tectorum* competition on very fine root production. Specific root length (m g^{-1}) also had a significant interaction of Seed source**Bromus tectorum**Timestep. Commercial seed had higher specific root length of all roots at 60 and 90 months of growth, but not at 30 and 120 days.

Discussion

Our results indicate significant shifts in plant traits between original farmed seed and seed collected after planting in disturbed fields with high *Bromus tectorum* cover. Plants from original, unselected commercial seed differed in plant traits from field collected seed, as found in earlier common garden experiments (Kulpa, 2010). Plants from the field selected seeds had smaller above and below ground biomass and produced smaller total root length and fine root length. The field selected seed produced a slightly lower proportion of fine roots to total roots compared to commercial seed. As a fraction of plant, the field selected seeds produced higher amounts of root weight relative to total plant size.

Other processes could be responsible for the observed shifts in plant traits in field seed sources, but processes other than natural selection are unlikely. Biased plant selection in the field could be responsible for the observed shifts between commercial and field established seed. Seeds were collected from 100 random plants that established at each site, from plants that were at least 0.50 meters from one another. Plants for field seed collection were easily identified as those that were planted during the restoration seeding (Kulpa, 2010). Seeds for the pots study were from 15 randomly selected plants of the 100 from the field. Thus, we do not have evidence for a biased field sampling design as the cause of the significant shifts in plant traits. Genetic drift could also result in a trend toward smaller plants, but the same trend toward smaller

plants in both field sites suggests that random processes of drift are not responsible for the trend.

This study does not provide evidence for selection for root traits related to competitive water and nitrogen acquisition. The shift to lower proportion of fine roots in field selected seed was not expected because fine root length is very important in nutrient uptake (Nye & Tinker 1977). Fine roots production has been shown to be important for uptake of immobile nutrients such as phosphorus (Silberbush & Barber 1983) but may be of less important in the uptake of water due to diffusion through the soil. Fine root production represents a substantial investment of carbon, nitrogen, and energy. One tradeoff of producing fine roots is that although they are important for accessing immobile nutrients, fine roots have shorter lifespan (Eissenstat *et al.* 2000) and are more susceptible to degradation than roots of larger diameter and thus may not represent an efficient investment of plant resources.

The shift to smaller plants suggests that the selective agent for the observed shifts is limited water and nutrients. Larger plants need more water and nutrients for establishment and survival, and leaf area cannot be supported unless sufficient water is available for transpiration. Although we did not experimentally manipulate *Bromus tectorum* cover and water in restoration areas, *Bromus tectorum* is known to have a large effect on the water status of invaded fields (Cline *et al.* 1977; Melgoza, Nowak & Tausch, 1990) and it is likely that *Bromus tectorum* was able to take up available water

and nutrients early in the growing season and only small *Elymus* plants with low water and nutrients demand were able to establish. The selection for smaller plants was observed in both seed collections from restoration areas, thus the phenomenon is not an isolated occurrence.

Field selected seed did not have a shift in competitive ability against *Bromus tectorum*, thus we do not reject null hypothesis 2. *Bromus tectorum* presence had similar proportional negative effect on production amongst seed sources. *Bromus tectorum* did have a significant effect on all plant traits and differences in competitive response amongst seed sources would have been induced if there truly were differences. It does not appear as though direct competition is the causal agent for shifts toward smaller plants with higher investment in root biomass observed in this study. Alternatively, there may not be variation in competitive traits within commercial seed for selection to act upon, but our data suggests that sufficient variation is present in commercial seed.

We do not provide concrete evidence for *Bromus tectorum* being a selective force on *Elymus* plant traits in the field, but indirect effects of *Bromus tectorum* on the soil environment may be selecting for smaller plants. The study does suggest that selection for smaller plants with higher relative root biomass does occur during establishment of *Elymus* seeds. To test whether *Bromus tectorum* causes the selection or the abiotic environment alone can account for changes in plant traits would require a

field establishment study in which replicated plots with *Bromus tectorum* presence and absence was manipulated as *Elymus* plants were establishing and measurements of the seedbed microenvironment taken.

This work does support the thesis that novel species interactions involving invasive species can cause evolutionary changes in native species. However, the rate and extent of such changes is not known. Population dynamics depend on establishment, but variable seed output and investment in reproduction at different points in a plant's life can influence demography. The population dynamics of *Elymus* in natural settings are not the same as seeding in restoration areas. *Elymus* in natural settings produces varying amounts of seed each year, and some are able to establish amongst mature plants, but variation in year to year conditions may result in different directions of selection in different years. Shift in traits of a mean population composed of plants that established at different times could cause a mosaic of plants with different traits and not necessarily consistent selection for smaller plants. If there was a selection for establishment of seeds of smaller plants but small amounts of establishment each year, shifts may occur in plants that established after *Bromus tectorum* cover was high, but it would be a long time before the total population could be affected.

Selection for smaller plants in fields with *Broums tectorum* cover has implications for plant selection and breeding for restoration plant materials. We found evidence that establishment of restoration plants in the Great Basin depends on the ability to survive

under limited resource conditions imposed by *Bromus tectorum*. Selection of plants that are able to establish in fields and survive to reproductive maturity should be the primary goal. Competitive ability can be measured in various ways but is most often measured with biomass or production of plants in competition relative to plants in monoculture (Aarssen & Keogh 2002). Small plants are competitive because they establish and are able to reach reproductive maturity, thus they have higher fecundity than large plants.

A suite of traits related to acquiring limiting nutrients was not important in seedling establishment native Great Basin grasses, but might be important in competitive effect as mature plants. It is unknown whether ability of seedlings to tolerate *Bromus tectorum* competition is correlated with *Bromus tectorum* suppression as adults. Plant restoration materials should be screened and selected for competitive response to *Bromus tectorum* during establishment and competitive effect on *Bromus tectorum* as established plants. Selecting seed from reproductive plants that are able to tolerate direct and indirect effects of *Bromus tectorum* is a good first step, but direct testing of the ability of the seed to establish should be a standard practice for selection of plant restoration materials.

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Tables

Table 1. ANOVA results for response variables of *Elymus elymoides* plants from original commercial seed and seed from plants that established in the field post-restoration seeding. *Elymus* plants were planted with and without competition by *Bromus tectorum* in a completely randomized design.

Response Variable	Effect	NumDF	DenDF	FValue	ProbF
Length	Seed source	2	42	46.30	0.0000
	Seed source* <i>Bromus tectorum</i>	2	1519	2.93	0.0538
	Seed source* <i>Bromus tectorum</i> *Timestep	6	1519	1.75	0.1068
Length of fine roots (0.10-0.20 mm diameter)	Seed source	2	42	47.69	0.0000
	Seed source* <i>Bromus tectorum</i>	2	1519	1.22	0.2945
	Seed source* <i>Bromus tectorum</i> *Timestep	6	1519	1.75	0.1051
Length of very fine roots (0.05-0.10 mm diameter)	Seed source	2	42	51.66	0.0000
	Seed source* <i>Bromus tectorum</i>	2	1519	4.78	0.0086
	Seed source* <i>Bromus tectorum</i> *Timestep	6	1519	2.19	0.0412
Average diameter	Seed source	2	42	1.88	0.1656
	Seed source* <i>Bromus tectorum</i>	2	1519	1.07	0.3441
	Seed source* <i>Bromus tectorum</i> *Timestep	6	1519	1.25	0.2769
Root system surface area	Seed source	2	42	37.05	0.0000
	Seed source* <i>Bromus tectorum</i>	2	1519	3.14	0.0436
	Seed source* <i>Bromus tectorum</i> *Timestep	6	1519	1.37	0.2224
Specific root length	Seed source	2	42	21.52	0.0000
	Seed source* <i>Bromus tectorum</i>	2	1516	1.52	0.2192
	Seed source* <i>Bromus tectorum</i> *Timestep	6	1516	3.39	0.0025
Root weight	Seed source	2	42	14.18	0.0000
	Seed source* <i>Bromus tectorum</i>	2	1534	2.25	0.1057
	Seed source* <i>Bromus tectorum</i> *Timestep	6	1534	1.61	0.1416
Shoot weight	Seed source	2	42	46.48	0.0000
	Seed source* <i>Bromus tectorum</i>	2	1532	2.51	0.0813
	Seed source* <i>Bromus tectorum</i> *Timestep	6	1532	1.92	0.0751
Root fraction of total plant weight	Seed source	2	42	19.77	0.0000
	Seed source* <i>Bromus tectorum</i>	2	1530	1.24	0.2886
	Seed source* <i>Bromus tectorum</i> *Timestep	6	1530	0.86	0.5257
Fine root fraction of total length	Seed source	2	42	7.57	0.0016
	Seed source* <i>Bromus tectorum</i>	2	1519	1.65	0.1923
	Seed source* <i>Bromus tectorum</i> *Timestep	6	1519	1.90	0.0774

Figures

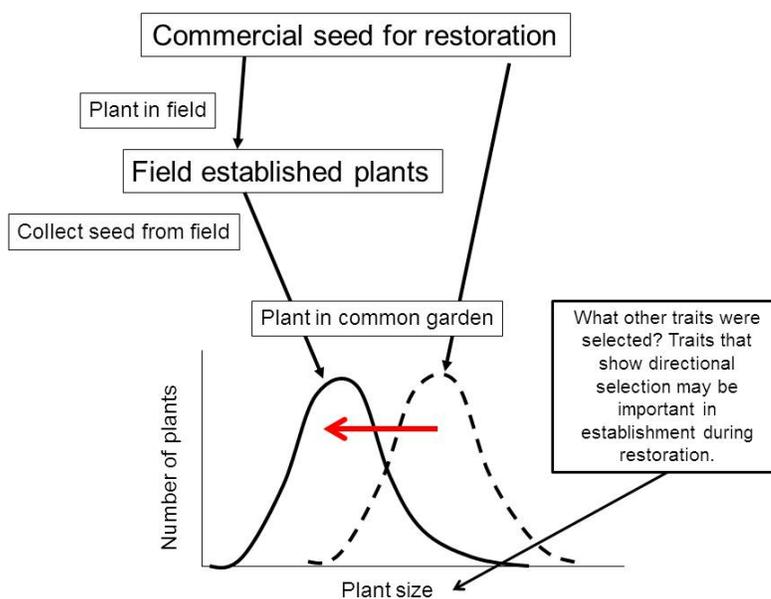


Figure 1. Diagram showing the expected distribution of plant traits after selection during a restoration planting. The diagram illustrates a mean shift in plant size indicating a selection for smaller plants in the field. Previous data has indicated directional selection for smaller overall plant size aboveground. Root traits related to nutrient acquisition and belowground competition are expected to show directional shifts as well.

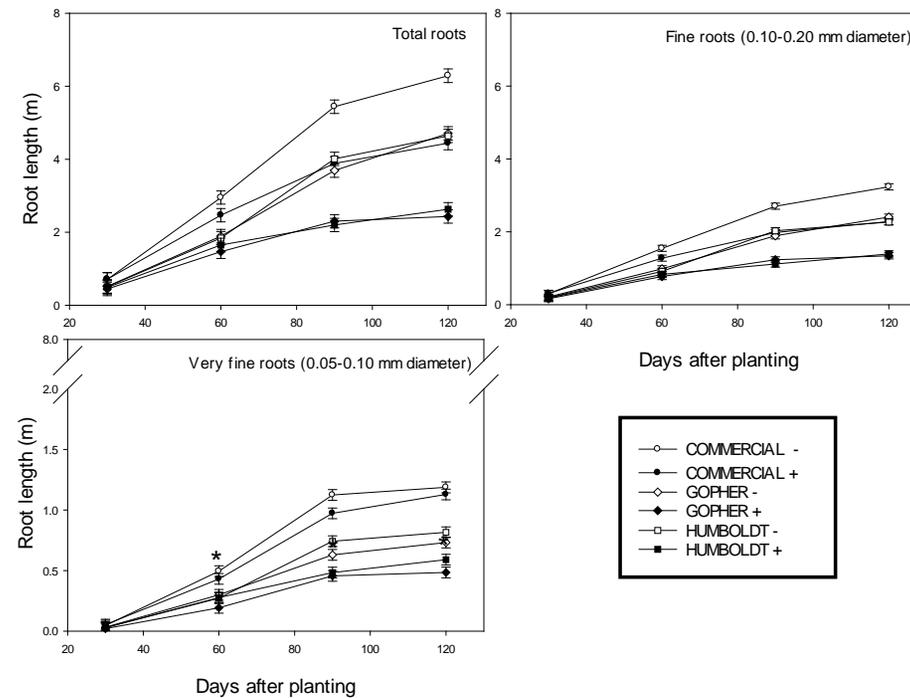


Figure 2. Root length of *Elymus elymoides* plants in monoculture (open circles, -) and competition (filled circles, +) with *Bromus tectorum*. The left panel shows total root length, the middle panel shows the length of roots in the 0.10-0.20 mm diameter class (fine roots), and the right panel shows the length of roots in the 0.05-0.10mm diameter class (very fine roots). Note the break and different pre-break scale on the very fine root panel from the other panels. For the total length of roots and the length of fine roots *Bromus tectorum* had a significant effect, but the interaction of Seed source and *Bromus tectorum* competition was not significant, meaning that all seed sources were negatively affected by *Bromus tectorum* to a similar extent. Seed sources differed in the total length of root produced, and seeds collected from the field after restoration planting produced less roots than the original commercially available seed. For very fine roots, the interaction of Seed source**Bromus tectorum* competition* Timestep was significant, and commercial seed had a smaller relative decrease in very fine root length between monoculture and competition pots at 60, 90, and 120 days of plant growth.

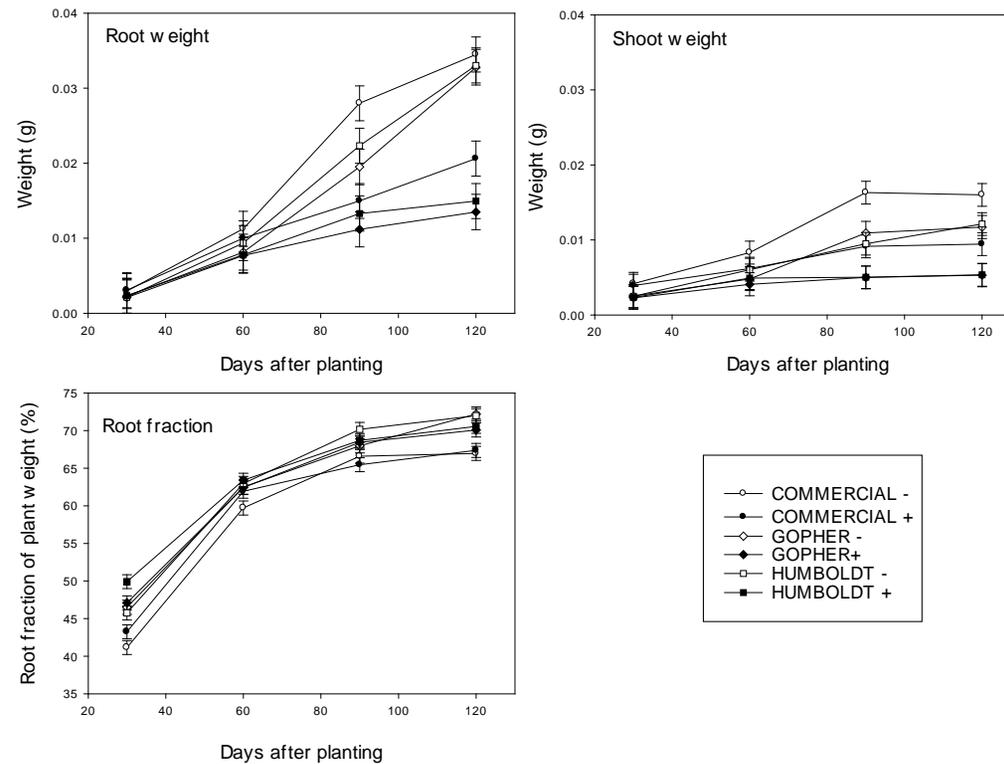


Figure 3. Root system weight, shoot system weight, and root fraction of total plant by weight for commercial seed and field collected seed from 2 field sites. Plants were grown with (filled circles, +) and without (closed circles, -) competition with *Bromus tectorum*. Root weight and shoot weight had a significant difference amongst seed sources: Commercial seed produced consistently larger plants. Root fraction of plant had a significant seed source effect: field seed had a higher fraction of root biomass.

Conclusions and future directions

Theories of ecosystem invasibility have been difficult to generalize at broad scales and across taxonomic groups (Lonsdale 1999). A commonly hypothesized mechanism of plant invasion is that invaders are more competitive than natives (Elton 1958; Sakai *et al.* 2001; Vila & Wiener 2004), and the mechanism has applicability in areas where competition determines fecundity. Competitive ability among plants in resource limited environments determines their ability to grow and reproduce. Two aspects of resource competition confer advantage to invasive species: (1) the ability to suppress neighbors through limiting available resources (“competitive effect”); and (2) the ability to avoid suppression (tolerance) via acquisition of resources in the presence of competing plants (“competitive response”, Goldberg & Landa 1991). Competitive effects in arid regions are induced through root system development and uptake of water (Fonteyn & Mahall 1978; Robberecht, Mahall & Nobel 1983; Ehleringer 1984). Competitive tolerance may be achieved through drought avoidance or high water use efficiency. With finite resource pools, both the competitive tolerance and effect are related: acquiring limiting nutrients both avoids suppression and decreases the available resource pool of the competitor. Competitive tolerance and effect are important during different parts of the plant life cycle (Fowler 1986). Competitive tolerance is an important part of the plant establishment phase as seedlings acquire enough resources to germinate, produce roots, and become photosynthetically carbon positive (Ross &

Harper 1972). After plants have established, mature plants that are able to induce a competitive effect on plants competing for a common resource pool have higher success (Humphrey & Schupp 2004).

Plant life history traits have been linked to invasiveness, but the relationship is not always clear and may depend on the habitat invaded and the biota present (Kolar & Lodge, 200; Sakai *et al.* 2001). Plants have been broadly categorized by their life history traits, and the interaction of life history traits and ecosystem characteristics influence plant success. Grime (1977) developed a framework in which he suggests three primary strategies of plants interact with the environment: the competitive strategy (low stress and low disturbance), stress-tolerant strategy (high stress and low disturbance), and ruderal strategy (low stress and high disturbance). Ecosystems have varying levels of interacting importance of stress, disturbance, and resources available for competition and these factors interact in species invasions (Thuiller *et al.* 2006). In un-invaded, undisturbed landscapes of the Great Basin, vegetation dynamics are driven by plant strategies of stress tolerance and conservation of water and nutrients rather than by ability to recover from disturbance or compete for nutrients released during disturbance. Increased disturbance and introduction of ruderal plants may have shifted the dominant strategy to a competitive one as resources become available through disturbance.

The change from a stress tolerant to a ruderal plant dominated ecosystem has implications for successful resource competition strategies (Diaz *et al.* 2004). Intact ecosystems with mature plants are relatively resistant to invasion (Chambers *et al.* 2007) and mature native grasses are able to suppress *Bromus tectorum* through uptake of resources in intact communities. Disturbance allows *Bromus tectorum* to effectively compete for unused resources and prevent native grass re-establishment (Melgoza, Nowak & Tausch 1990; Beckstead & Augsburger 2004). Increased frequency of fire, grazing, and human disturbances has shifted the ecosystem to favoring the ruderal plant life history strategy as resources are temporally available (water in the winter and early spring). Annuals generally have higher root nitrogen, rapid root growth rate, high nutrient uptake rates, and higher root respiration than perennials (Roumet, Urcelay & Diaz 2006). In a comparative study of plants with different life histories, annuals grew faster and produced a more branched root system than perennials (Gross, Maruca & Pregitzer 1992). Native perennial grasses are naïve to the strategy of rapid acquisition of nutrients following disturbance, and changes to the disturbance regime and introduction of annual plant may be key aspects of the observed invasion.

If a change to ruderal strategy of resource acquisition is driving the competition and success of plants, there are consequences for evolution and restoration. Native plants that are able to establish and survive in disturbed areas that have been invaded by annual plants may be those that employ a ruderal nutrient acquisition strategy as

seedlings in order to compete or may be those that can simply establish and survive under pressure by invasives. Chapter 1 suggests that the rapid acquisition of seasonally-available water by perennial plants is important in *Bromus tectorum* suppression. Chapter 2 results suggest that the application of native grasses in restoration sites results in selection for heritable traits in the establishing restoration plants, and that selection was for smaller plants with a relatively higher investment in root biomass. Smaller plants have lower water and nutrient requirements for growth and reproduction, thus they are able to survive on low amounts of resources. Both of these results suggest that acquisition of water by native plants is a critical factor in restoration in the Great Basin.

Genetic and phenotypic variability in native plants can influence the production of restoration materials. Heritable variation in traits that allow successful establishment may not be widely present in native plants, so selection and breeding may be unsuccessful or result in low genetic diversity of established natives. Additionally, it is not known if traits related to competitive tolerance as seedlings and competitive effect as mature plants are related, and if the traits are present in the same populations. Can plants be selected that have both the ability to establish in *Bromus tectorum* fields through low water demand and high root growth as seedlings and high suppression of *Bromus tectorum* through water use as mature plants? Further work is needed to

determine how much variation in these successful traits is present in commercial germplasms and how much variation is present in *Elymus* across the Great Basin.

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Appendix: additional figures from chapter 1

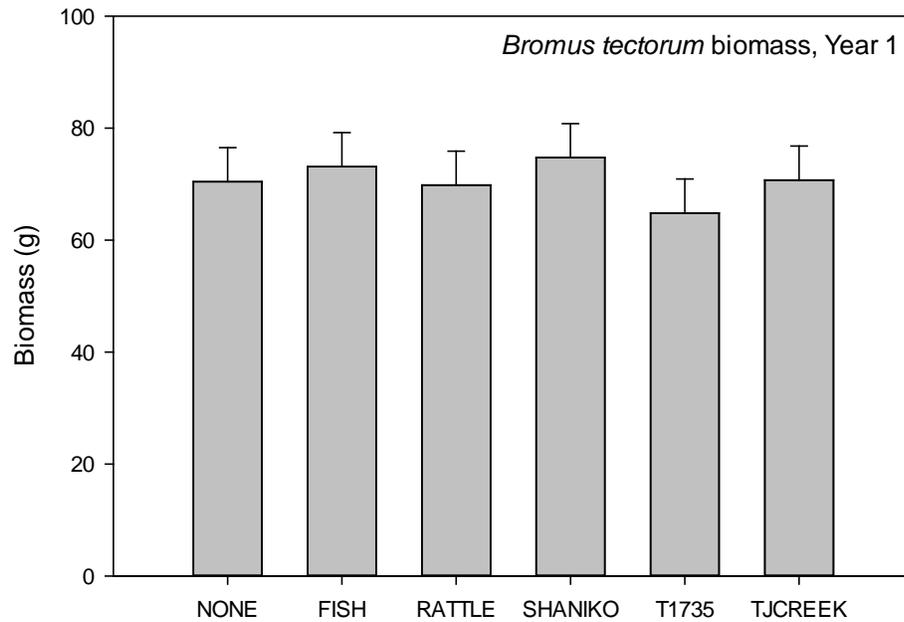


Figure 1. Biomass of *Bromus tectorum* in monoculture and competition with germplasms of *Elymus* in year 1 of the experiment. *Elymus* germplasms did not have a significant competitive effect on *Bromus tectorum*.

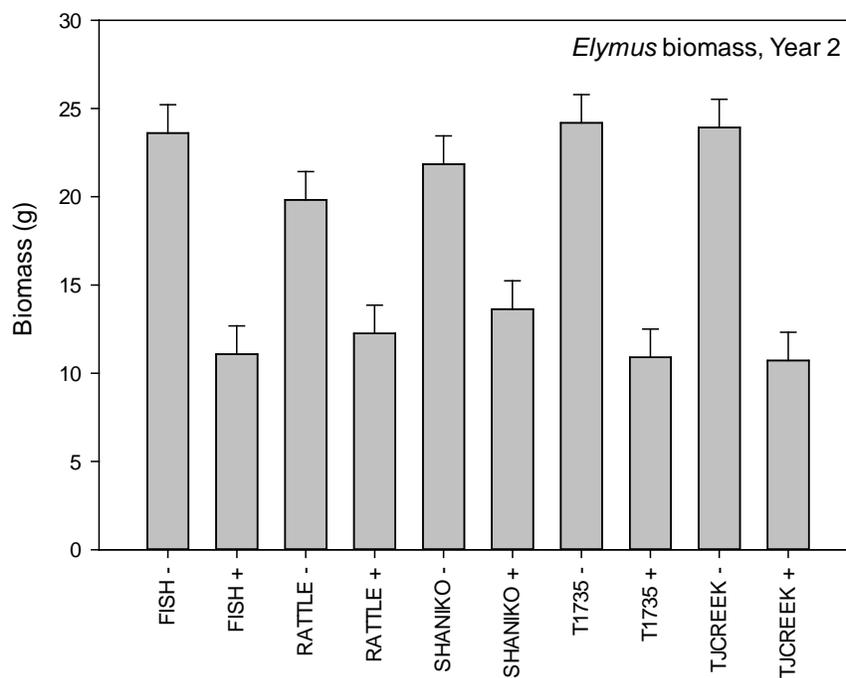


Figure 2. . Biomass of *Elymus* germplasms in monoculture (“GERMPLASM” -) and competition with *Bromus tectorum* (“GERMPLASM” +) in year 2 of the experiment. Germplasms responded similarly to *Bromus tectorum* competition (no significant Germplasm * *Bromus tectorum* interaction). *Bromus tectorum* had a significant negative effect on biomass of all *Elymus* germplasms.

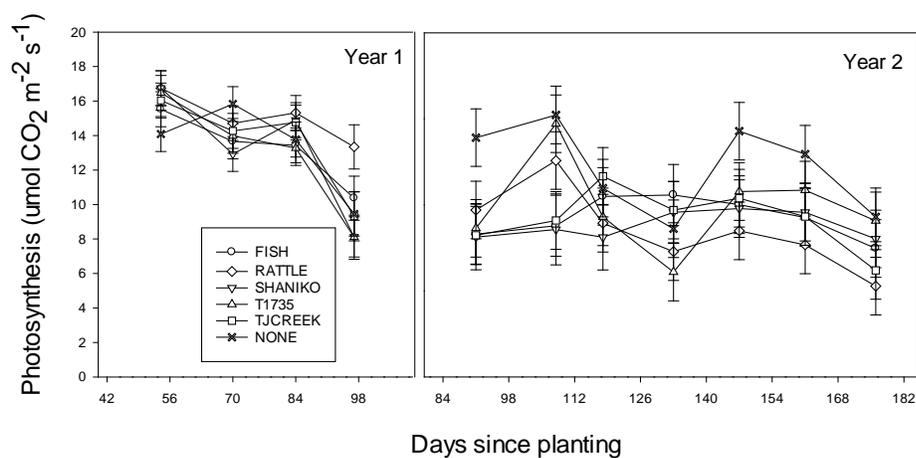


Figure 3. Photosynthesis of *Bromus tectorum* with and without competition with *Elymus*. Photosynthesis of *Bromus tectorum* was not significantly affected by the presence of *Elymus* in either year (Year 1: $f_{5,108}=1.91$, $P=0.099$; Year 2: $f_{5,240}=1.88$, $P=0.099$).