

University of Nevada, Reno

Effects of Seed Source on Seedling Performance of
Elymus multisetus (big squirreltail) in the Great Basin

A thesis submitted in partial fulfillment of the
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by

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ABSTRACT

Widespread invasion by *Bromus tectorum* L. (cheatgrass) has drastically altered native plant communities in the Great Basin. Certain native perennial grasses appear to be more tolerant of cheatgrass invasion and are therefore considered promising species for restoration in cheatgrass-invaded areas, including big squirreltail (*Elymus multisetus* M.E. Jones). Native populations may also be evolving in response to cheatgrass invasion. Genetic studies have shown high levels of inter- and intra- population genetic variation for grass species native to the Great Basin, but there is still a large knowledge gap in how this genetic variation can impact and be impacted by restoration. The goal of this research was to investigate the effect of seed source on restoration seedling performance in the Great Basin using *Elymus multisetus* as a case study, addressing the following questions: Can seeds from local-wild sources establish with greater success than those from regional-farmed sources? Are squirreltail seedlings from cheatgrass invaded sources more competitive than seedlings from uninvaded sources? Is it possible to identify growth traits that are advantageous for seedlings in cheatgrass invaded areas?

To answer these questions we conducted two separate common garden experiments. Differences that persist in a common garden likely have a genetic basis and can evolve via natural selection. Our first experiment investigated the effect of seed source on field establishment of big squirreltail by comparing seedling performance of locally-collected seed and commercially-produced seed from Oregon, Idaho, and California at a recently-burned site on the Hallelujah Junction Wildlife Refuge, Bordertown, CA. Several phenological and growth traits varied significantly between source populations. Eighty-six percent of local seeds emerged, compared to 71%, 61%

and 12% of seeds from Idaho, Oregon, and California, respectively. Local seeds emerged nine days earlier than other seeds sources on average. Through the first year, 7.6% of the local seedlings survived, followed by 5.2%, 4.8%, and 0.6% survival of Idaho, Oregon, and California seedlings, respectively. Though survivorship was highest for local seed, local seedlings produced 24% fewer leaves than the most productive seedlings from the Idaho seed source. These data would suggest that seed source is an important factor in seedling establishment and performance. If local seed can survive significantly better than regionally farmed seed, it may be both economically and ecologically beneficial to use seeds collected from relatively local sources in revegetation.

To examine the potential effect of cheatgrass invasion on the evolution of adaptive traits, our second experiment was conducted in a greenhouse and involved destructive harvesting of big squirreltail seedlings from invaded and uninvaded areas in order to measure both root and shoot growth traits. We determined if growth traits of plants from invaded areas displayed shifts consistent with evolution in response to cheatgrass invasion, measured genetic variation of potentially adaptive traits, and used a correlative approach to identify traits that may confer an advantage to native plants growing in cheatgrass invaded areas. Seedlings from invaded areas exhibited greater tolerance of competition and a greater ability to suppress cheatgrass than seedlings from adjacent uninvaded areas. Competitive ability was correlated with 10 day root:shoot ratio, root forks, and fine root length, but only 10 day root forks appear to be inherited and none have significantly shifted across invasion status. Additionally, we surveyed traits that varied between invaded and uninvaded areas. Invaded plants were smaller, allocated more biomass to roots, and produced a higher percentage of fine roots. Root

traits, including early root growth, may be an important component of competitive ability in *Elymus multisetus*. The ability of native populations to evolve in response to invasion has significant implications for management and restoration of cheatgrass-invaded communities.

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INTRODUCTION

Widespread invasion by *Bromus tectorum* L. (cheatgrass) has drastically altered native plant communities in the Great Basin. The conversion of approximately 12.7 million hectares of rangelands from communities dominated by native perennials to cheatgrass is one of the most severe terrestrial ecosystem changes in North America (Mack 1981; D'Antonio and Vitousek 1992; Menakis et al. 2003). Positive feedback exists between disturbance, cheatgrass invasion, and increased wildfire fire frequency, the combination of which leads to the continued decline of many native perennial species in cheatgrass-invaded areas (Young and Evans 1972; Evans et al. 2001; Brooks et al. 2004; Chamber et al. 2007). Traditional post-fire management is often insufficient to prevent the conversion of previously uninvaded areas to cheatgrass-dominated communities (Pellant 2004). Although revegetation with non-native species continues to have strong support (Asay et al. 2001; Thompson et al. 2006), land managers have also begun to pursue restoration of native perennial populations as a method of breaking the cheatgrass-altered fire cycle (Richards 1998; BLM 2001).

Certain native perennial grasses appear to be more tolerant of cheatgrass invasion and are therefore considered promising species for use in cheatgrass-invaded areas, including big squirreltail (*Elymus multisetus* M.E. Jones) and bottlebrush squirreltail (*E. elymoides* (Raf.) Swezey) (Hironaka and Tisdale 1963; Booth et al. 2003; Leger 2008). Two commercial germplasm releases of *Elymus elymoides* and one release of *E. multisetus* have been developed for use in restoration in the Great Basin (Jones et al. 1998, 2004a,b). It has been hypothesized that squirreltail may be able to compete with cheatgrass because it exhibits growth and phenology traits commonly associated with

early seral species (Young and Evans 1977; Jones 1998; Redente et al. 1992). However, in direct comparisons of growth traits between squirreltail and annual grass invaders, the invader usually outperforms squirreltail (Arredondo et al. 1998; Young et al. 2003; Young and Mangold 2008), suggesting that further research is necessary to explain squirreltail's ability to tolerate cheatgrass invasion.

Restoration of native perennial plant populations in the Great Basin can be both difficult and contentious. Seeding cheatgrass-invaded areas with native seed often results in low establishment and survival (Campbell and Swain 1973; Monsen and McArthur 1995; Thompson et al. 2006). As managers and scientists seek explanations for limited restoration success at arid sites, there are a variety of theories presented: high annual variation in precipitation (Bleak et al. 1965); failure to address underlying ecosystem processes; competition from extant vegetation (Roundy and Call 1988); vulnerability of seedlings to microsite variation (Evans and Young 1972); or inappropriate seed material. All of these factors probably play some role in limiting establishment.

There are widely divergent opinions about how changes in the genetic identity of native seed could improve restoration seedling establishment. Some advocate that the use of commercially available seed, which is often collected from a single population, sometime even from a single genotype, and farm-grown hundreds of miles from a project site, lacks both appropriate quality and quantity of genetic variation, and that locally-collected seed could improve seedling establishment (e.g. Guinon 1993; Meyer and Monson 1993; Burton and Burton 2002). It is also argued that the introduction of non-local seed may be deleterious to the fitness and evolutionary potential of existing native populations (Montalvo et al 1997; McKay et al. 2005). Conversely, others believe that

local seed material may not be well-adapted for use in highly disturbed areas and that the manipulation of commercially available seed can produce superior tolerance of common biotic and abiotic stresses (Jones and Monaco 2009).

Genetic variation and the potential for local adaptation of seed material receive considerable attention in restoration studies (Rice & Knapp 1994; Dyer & Knapp 1998; Hufford and Mazer 2003; Broadhurst et al. 2008), but there have been few field-based explorations of their impacts in the Great Basin. Humphrey and Schupp (2002) compared local and commercial seed sources of seven native species in the Great Basin and found that local seeds showed first and second year survival advantages over commercial seed of four species, but the advantage had disappeared by three years. For many native Great Basin species, traits that affect seedling survival differed between source populations when examined in a common environment (e.g. Meyer and Monsen 1991, 1992; Jones et al. 2003). Genetic studies have shown high levels of inter- and intra- population genetic variation for grass species native to the Great Basin (e.g. Larson et al. 2001, Larson et al. 2003). Reciprocal transplants have shown relatively small scales of local adaptation for some species (e.g. McArthur et al. 1983; Link et al. 2003) but inconclusive results for others (e.g. Young and Evans 1990; Wang et al. 1997)

There is still a large knowledge gap in how the genetic variation evident in many native Great Basin species can both impact and be impacted by restoration. Understanding the factors that may affect restoration of native populations is a major research priority for the Great Basin (Chambers and Wisdom 2009). There are so many aspects of this question to tackle that it is difficult to know where to start; our choice was driven by related evolutionary biology research underway in the Leger Lab that

demonstrated performance differences between plants from cheatgrass invaded areas and adjacent uninvaded areas that are consistent with rapid evolutionary change (Leger 2008; Goergen, Leger, and Espeland in review). Evidence is emerging that biological invasions can produce rapid evolutionary change in native plant populations (e.g. Callaway et al. 2005; Lau 2007; Meador and Hild 2007; Leger 2008). The ability of native populations to evolve in response to cheatgrass invasion would have significant implications for the management and restoration of cheatgrass-invaded communities.

The goal of this research was to investigate the effect of seed source on restoration seedling performance in the Great Basin using *Elymus multisetus* as a case study, addressing the following questions: Can seeds from local-wild sources establish with greater success than those from regional-farmed sources? Are squirreltail seedlings from cheatgrass invaded sources more competitive than seedlings from uninvaded sources? Is it possible to identify growth traits that are advantageous for seedlings in cheatgrass invaded areas?

To answer these questions we conducted two separate common garden experiments. Differences that persist in a common garden likely have a genetic basis and can therefore be subject to natural selection (Turesson 1922; Clausen et al. 1947; Endler 1978). Our first experiment investigated the effect of seed source on field establishment of big squirreltail by comparing seedling performance of locally-collected seed and commercially-produced seed from Oregon, Idaho, and California at a recently-burned site on the Hallelujah Junction Wildlife Refuge, Bordertown, CA. To examine the effect of cheatgrass invasion on the evolution of adaptive traits, our second experiment was conducted in a greenhouse and involved destructive harvesting of seedlings from invaded

and uninvaded areas in order to measure both root and shoot growth traits. We determined if growth traits of plants growing in invaded areas displayed shifts consistent with evolution in response to cheatgrass invasion, measured genetic variation of potentially adaptive traits, and used a correlative approach to identify traits that may confer an advantage to native plants growing in cheatgrass invaded areas. As a final note, this thesis employs the University of Nevada Reno's alternative thesis format; chapters are formatted for journal publication and follow the formatting guidelines of the journal of submission.

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**Seed source affects seedling establishment of *Elymus multisetus* (big squirreltail)
in post-fire revegetation in the Great Basin**

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Ecological Restoration—Original Research Article Submission

ABSTRACT

Post-fire revegetation with native perennial grasses is difficult to achieve in disturbed arid rangelands. If local populations are adapted to current conditions, then locally-collected seed is predicted to have higher survival than non-local seed and using local seed should improve revegetation success. However, sufficient quantity of local seed is often difficult to obtain commercially. For revegetation projects in the Great Basin, big squirreltail (*Elymus multisetus*) seed material often originates from source populations that are hundreds of miles from the project site. We investigated whether seed source affected first year establishment of big squirreltail seedlings in a common garden field trial 30 miles north of Reno, NV, using locally-collected seed and commercially-produced seed from Oregon, Idaho, and California. Several phenological and growth traits varied significantly between source populations. Eighty-six percent of local seeds emerged, compared to 71%, 61% and 12% of seeds from Idaho, Oregon, and California, respectively. Local seeds emerged nine days earlier than other seeds sources on average. Through the first year, 7.6% of the local seedlings survived, followed by 5.2%, 4.8%, and 0.6% survival of Idaho, Oregon, and California seedlings, respectively. Though survivorship was highest for local seed, local seedlings produced 24% fewer leaves than the most productive seedlings from the Idaho seed source. These data would suggest that seed source is an important factor in seedling establishment and performance. If local seed can survive significantly better than regionally farmed seed, it may be both economically and ecologically beneficial to use seeds collected from relatively local sources in revegetation.

KEYWORDS: arid rangeland revegetation; big squirreltail; *Elymus multisetus*; Great Basin; local adaptation

INTRODUCTION

Reciprocal transplant and common garden studies have repeatedly shown that plant populations can be highly adapted to local environmental conditions (Clausen et al. 1940; Fenster and Galloway 2000; Joshi et al. 2001, Leimu and Fisher 2008). This leads to the expectation that, when seed material is chosen for a revegetation project, seeds collected near the project site should yield higher survival than commercially produced seed material from distant sources (Knapp & Rice 1994), a hypothesis supported in field tests (e.g. Cotts et al. 1991; Petersen 1994; Gustafson et al. 2004; Rice & Knapp 2008). There is now widespread support for the use of locally-collected native seed in restoration (Linhart 1995; Hufford and Mazer 2003; Broadhurst et al. 2008) and the preservation of local genetic variation in restored plant populations has become a priority for many land managers (Buck et al. 1970, Guinon 1993; Meyer and Monsen 1993; Richard et al. 1998; Burton and Burton 2002). Both the National Park Service and US Forest Service have instituted seed policies that encourage the use of either local seed or seed from similar environmental conditions to the proposed revegetation site (NPS 1993; USFS 2008).

In addition to concerns about reduced performance, there is also concern that the use of non-local restoration seed may reduce *in situ* genetic variation in natural populations (Montalvo et al. 1997; McKay et al. 2005). Commercially available restoration seed is often derived from a single source population and sometimes even

from a single genotype (Young et al. 2003; Jones and Larson 2005, Shaw et al. 2005). Inadvertent selection can occur in the collection and propagation of restoration seed and alter genetic variation (Campbell and Sorensen 1984; Meyer and Kitchen 1994). There are examples of outbreeding depression reducing fitness in native plant populations (Montalvo and Ellstrand 2001; Waser and Price 2000), but not specifically as a result of restoration. On the whole, the impact of large-scale use of non-local seed on the genetic integrity of native plant populations remains poorly understood (Knapp and Dyer 1998).

Why isn't local native seed used in all restoration attempts? First, local seed from native species can be difficult to obtain in sufficient quantity for large-scale projects and is considerably more expensive than commercially available alternatives (Roundy et al. 1997; Shaw et al. 2005). Second, the preservation of local genetic variation may not guarantee that local seed material performs optimally at restoration sites. Highly adapted populations may also be at greater risk of maladaptation in changing conditions (Crespi 2000). If conditions have changed from those under which a local population has evolved, which can be the case in disturbed ecosystems, then the population may not possess sufficient genetic variation to produce a successful phenotype in the new environment (Rice and Emery 2003; Jones and Monaco 2009). Furthermore, the success of some invasive species demonstrates that nonlocal genotypes can effectively compete in novel environments without significant genetic differentiation or local adaptation (Williams et al. 1995).

Genetic variation and local adaptation of seed material receive considerable attention in restoration studies but there have been few field-based examinations of their impacts on restoration of cheatgrass (*Bromus tectorum* L.) degraded rangelands in the

Great Basin (e.g. Humphrey and Schupp 2002). The conversion of Great Basin rangelands from native perennial grasses to cheatgrass is one of the most severe ecological degradations in the United States (Mack 1981; D'Antonio and Vitousek 1992). By 2000, approximately 12.7 million hectares of the Great Basin had been converted to cheatgrass (Menakis et al. 2003), and an estimated 45% of the Great Basin is at risk of conversion (Bradley and Mustard 2005). It has long been known that cheatgrass increases both the frequency and extent of wildfires in the Great Basin (Pechanec and Hull 1945). As a cool-season annual grass, cheatgrass matures earlier than native species and provides easily ignited fuels that promote rapid rate of fire spread (Stewart and Hull 1949, Young et al. 1987). It is an efficient user of nitrogen and soil water, has large ecological amplitude, and tolerates moderate to heavy grazing (Young and Evans 1972; Rice and Mack 1991; Daubenmire 1992). By altering both nutrient cycles and fire regimes, cheatgrass has wrought extensive ecological degradation (Evans et al. 2001; Brooks et al. 2004). Invasion can sometimes exclude native perennial vegetation (Young and Evans 1972).

During the summers of 1999 and 2000, wildfires burned a record 2.7 million acres of rangeland in the Great Basin (BLM 2000, 2001). The advent of two consecutive severe fire years spurred federal land managers to acknowledge the factors contributing to a higher frequency of large-scale fires and to evaluate strategies for managing this altered fire regime (BLM 2001). In 2000, the Bureau of Land Management (BLM), which manages nearly 60% of the Great Basin, acknowledged that traditional post-fire rehabilitation is not sufficient to tackle the ecological problems associated with wildland fires, resolving that restoration was necessary (BLM 2000). Traditional post-fire

rehabilitation focused on soil stability and used mainly non-native grasses, particularly crested wheatgrass (*Agropyron cristatum* L.) (Pellant 2004). In its Great Basin Restoration Initiative, the BLM endorsed the use of native seed for post-fire revegetation (BLM 2000).

Big squirreltail (*Elymus multisetus* M.E. Jones) and bottlebrush squirreltail (*E. elymoides* (Raf.) Swezey) are native perennial bunchgrasses that are considered promising species for use in restoration in cheatgrass infestations (Jones 1998; Richards et al. 1998). The two species are very closely related and considered conspecific by some taxonomists (Holmgren and Holmgren 1977; Arnow 1993; Larson et al. 2003). They are often treated as a species complex, as they will be here (Jones 1998). Squirreltail has a demonstrated ability to persist alongside cheatgrass (Hironaka and Tisdale 1963; Booth et al. 2003; Leger 2008). Relative to other perennial grasses, squirreltail may be able to compete with cheatgrass because it exhibits growth and phenology traits similar to early seral species. It rapidly reaches reproductive maturity, exhibits high germination and growth rates, has relatively high specific leaf areas and root lengths, and high nutrient use efficiency (Young and Evans 1977; Redente et al. 1992; Arredondo et al. 1998; Young et al. 2003). Squirreltail also has a high tolerance to repeated burning relative to other native perennial grasses (Wright 1971; Britton 1990). Two commercial germplasm releases of *Elymus elymoides* and one release of *E. multisetus* have been developed for use in restoration in the Great Basin (Jones et al. 1998, 2004a,b).

Squirreltail populations exhibit both high genetic and phenotypic variation across the Great Basin, indicating that the variation required for local adaptation exists (Jones et al. 2003; Larson et al. 2003). However, recent studies present somewhat contradictory

information about the effect of population level genetic variation on traits in Great Basin *Elymus* species (Humphrey and Schupp 2002; Larson et al. 2003; Robins and Jenson 2008; Rice et al. 2009). For thickspike wheatgrass (*Elymus laceolatus* (Scribn. and J.G. Sm.) Gould), seed yield is an inherited trait, while biomass production is not (Robins and Jenson 2008). Humphrey and Schupp (2002) compared local and commercial seed sources of bottlebrush squirreltail and found that local seeds showed first and second year survival advantages over commercial seed, but the advantage had disappeared at three years. In the neighboring Sierra Nevada, reciprocal transplants of bottlebrush squirreltail demonstrated fitness differences that varied with 300m elevation differences in collection site as well as aspect, with local seed outperforming non-local seed (Rice et al. 2009).

Our goal was to evaluate whether seedling establishment in post-fire revegetation is affected by seed source of *Elymus multisetus* by testing local and regional seed in a common garden experiment. Differences that persist in a common garden are likely have a genetic basis and therefore could evolve via natural selection (Clausen et al. 1940; Endler 1978). Many studies of local adaptation compare performance among populations by hand-planting seedlings, rather than sowing seeds, into common environments (e.g. Shaw 1986; Booth et al. 2003; Hufford et al. 2008). While this process results in higher survival rates, it obscures an important stage of survivorship—the transition from seed to seedling—and eliminates the opportunity to examine how emergence percentage and phenology affect performance. We used a precision seeding technique that allowed us to efficiently sow and track a large quantity of seeds and to ask the following questions: Does emergence and survivorship vary between seed sources in *Elymus multisetus*? If so,

what are the implications for our understanding of local adaptation in a species complex that holds both ecological and managerial importance in the Great Basin?

METHODS

In order to address the importance of seed source in revegetation, we conducted a common garden field experiment at the Hallelujah Junction Wildlife Refuge, near Bordertown, Sierra County, CA, approximately 30 miles north of Reno, NV. Elevation at the field site is ~1500m above sea level and slope ranges from 2-15%. The soil type is Trosi Very Stony Sandy Loam, with parent material consists mostly of alluvium derived from mixed bedrock (NRCS 2008). This soil type is considered nonsaline, exhibits very lower water holding capacity (0.00-0.254mm/hr), and has very low available water (~38.1mm) (NRCS 2008). Average temperature in January is 0.4°C and 21.3°C in July, with annual precipitation averaging 265.2mm (NCDC 2002). The site is managed by California Department of Fish and Game and has been rotationally grazed for over 50 years. Since a moderate intensity fire in 2007, the study area has not been grazed. The plant community is shrub-steppe dominated by mountain big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle and Young). The main graminoid components are Sandberg's bluegrass (*Poa secunda* J Presl. spp. *secunda*), big squirreltail, and cheatgrass. While cheatgrass exists at the site, there has not been wholesale conversion, as it seen in many other sites with extended grazing history in the Great Basin.

On 20 November 2008, we sowed 250 big squirreltail seeds from four seed sources (1000 seeds total) using a precision seeding method. Seed sources are listed below with reference name, generation status (G_x), collection site, and production site

(when applicable): Local source— G_0 collected in July 2008 from within 0.40 km of the study area (Balls Canyon, Sierra County, CA); California source— G_0 collected from Tehama County, CA and G_1 farm-grown in Yolo County, CA; Oregon source— G_0 collected from Crooked River National Grassland, Jefferson County, OR and farm-grown in Franklin County, WA (generation status of farmed seed unknown); Idaho Source— G_0 collected from Sand Hollow, Gem County, ID (Jones et al. 1998) and G_1 , G_2 , and G_3 farm-grown in Lehi, Utah County, UT. For regional-farmed sources, seeds were randomly selected from 1 kg bags. For the local population, seeds were randomly selected from a 2008 collection, bulked from ~100 individual plants. Seed viability was assessed by tetrazolium testing (described in Miller 2005), with four replicate treatments of ten seeds from each seed source (160 seeds total).

Each seed was individually weighed and then glued to a toothpick by its glume using Elmer's glue. Seeds on toothpicks were sown directly in the field in a complete random design with twenty-five rows of forty seeds each. This precision seeding technique allowed us to efficiently find and identify seedlings in the field. Each row was spaced 1m apart and seeds were spaced 0.5m apart within rows. Seeds were sown to a depth of ~2mm. Some toothpicks were physically touching an extant plant, and competition status (i.e. touching or not touching extant plant) was noted for each seed to facilitate analysis of possible competition effects.

Seed establishment and short-term seedling survivorship are commonly used as success measurements for restoration projects (Zedler and Calloway 2000; Ruiz-Jaen and Aide 2005). Seed emergence was tracked weekly from 20 November 2008 through 20 April 2009. Since *Elymus multisetus* is a cool-season perennial grass, plants

experience summer dormancy which can potentially complicate measurement of survivorship by masking dead plants. As such, the number of plants exhibiting active growth (i.e. green tissue) was used as a proxy for survivorship. Active growth was assessed in April, May, June, July, and September 2009 and again in January 2010. Pure live seed (PLS) was calculated by multiplying mean viability percentage (determined by tetrazolium test) and quantity sown. PLS was used to report adjusted emergence (PLS x % emerged) and survivorship (PLS x % active growth) percentages. We observed other perennial grasses senescing from late-June through late-July. A sharp decline in active growth in experiment plants in July was used to demark the end of the first growing season.

Measurements of leaf length and quantity were taken monthly from May to September 2009. Leaf length is strongly correlated with total biomass in perennial grasses (e.g. Scheiner 1989; Sjevcar 1990; Arrendendo et al. 1998; Leger 2008). It served as a proxy for biomass, so that we did not have to destructively harvest plants before seed set. Unfortunately, no plants produced seed in their first year. In May and June, a subsampling of leaf length measurements was taken from 20 randomly selected individual plants from each source (n=69; only 9 actively growing plants from California source). By July, the number of actively growing plants was severely reduced and censuses were conducted in July and September (n=133 and 24 respectively). Total leaf length was calculated by summing the lengths of all leaves exhibiting active growth in July.

All analyses were performed in JMP 7.0.2 (SAS Institute 2007). Emergence and active growth were classified as ordinal data and analyzed using logistic regression.

ANOVA was used to analyze the effects of seed source (fixed factor), seed weight (continuous covariate) and competition status (fixed factor) on emergence timing and July leaf length. The effects of seed source on seed viability and seed weight were analyzed using single-factor ANOVAs. The effects of emergence timing (fixed factor) on active growth and leaf length were also analyzed using single-factor ANOVAs. Significant results ($\alpha=0.05$) were analyzed *a posteriori* using the Tukey-Kramer HSD method. For seed weight and emergence timing, transformations were not required to meet assumptions of normality and homogeneity of variance for ANOVA, but leaf lengths were log-transformed to achieve a normal distribution. For the July leaf length analysis, the California source was excluded because only one plant was actively growing. Raw means, rather than least means squares, are reported for all measurements.

RESULTS

Seed weight varied significantly among seed sources ($P < 0.0001$, $F_{3,1000}=96.0$, $r^2=0.22$), with each source exhibiting a significantly different mean value in *a posteriori* comparison (Figure 1). The range of seed weights was narrow—0.001-0.007 grams—and there was substantial overlap in seed weight variation among seed sources.

Tetrazolium testing of seed viability revealed significant differences between seed sources ($P=0.0003$, $F_{3,16}=14.3$, $r^2=0.72$). Viability percentages were 100% for Oregon and local sources, 93% for Idaho, and 70% for California, with the California source significantly different than the remaining three in *a posteriori* comparison.

Emergence percentage varied significantly by seed source and seed weight (Table 1b). Local seeds exhibited the highest emergence percentage—86.4%, followed by

Oregon (71.2%), Idaho (56.8%) and lastly California (8.4%) (Figure 2). Accounting for seed viability, adjusted emergence increased to 12.0% for California and 61% for Idaho; local and Oregon sources were unchanged. Emergence percentage increased with increasing seed weight (Table 1b). Emergence percentage was not significantly affected by competition status at time of sowing (Table 1b), but there was a trend for neighbors to decrease emergence percentage (57.5% emergence without competition; 46.7% with competition).

Emergence timing (i.e. days to emergence) varied significantly by seed source (Figure 3) and competition status, but was not significantly affected by seed weight (Table 1a). Local seed emerged, on average, nine days earlier than other seed sources (Figure 3). Mean days to emergence for seeds experiencing competition was 111 days, compared to 102 days for those seeds without competition. Emergence timing also varied by seed weight, with heavier seeds emerging earlier; however, the relationship was not significant (Table 1a).

The number of plants exhibiting active growth was significantly different among seed sources at all six assessments (Table 1b, Figure 4). In April, May, and June, the greatest quantity of actively growing plants was from the local source (Figure 4). In July, plants from local and Oregon sources were nearly equal in abundance ($n_{\text{local}}=48$; $n_{\text{OR}}=51$), while both were more abundant than the other two sources ($n_{\text{CA}}=1$; $n_{\text{ID}}=8$). Oregon seed source exhibited the highest PLS-adjusted percentage of actively growing plants in July (20.4%), followed by local (19.2%), Idaho (14.3%), and California (0.6%) (Table 1b). There was a sharp decline in active growth in all plants through June and July (Figure 4). By September, total active growth was very low (<3%). By January, total active growth

increased slightly to 4.4% overall. The local seed source exhibited the highest adjusted percentage of actively growing plants in January (7.6%), followed by Oregon (5.2%), Idaho (4.8%), and California (0.6%) (Table 1b). At the end of the data collection period, there were only 44 actively growing plants out of 557 emergents sown from 1000 seeds; 43% (n=19) of all actively growing plants were produced from local seed. Neither seed weight nor competition status at time of sowing significantly predicted whether a plant would be actively growing at any measurement period (Table 1b). Active growth was significantly correlated to emergence timing in May, June, July, September, and January (data not shown; all $P < 0.02$), though not in April ($P=0.053$). For the last five assessments, plants exhibiting active growth tended to have earlier emergence dates.

Mean leaf length in July varied significantly by seed source, but not by competition status or seed weight (Table 1a). Seedlings from the Idaho seed source exhibited the greatest mean leaf length (22.9 ± 3.0 cm), mostly due to three very large outliers. Mean leaf lengths for California, Oregon, and local sources were 17.0 cm (n=1), 18.1 ± 2.4 cm, and 11.9 ± 2.5 cm respectively. After transformation and analysis, there were no significant differences between the Idaho and local sources or between the Idaho and Oregon sources, but differences between local and Oregon sources persisted. Mean leaf length was also correlated to emergence timing with earlier emerging seedlings exhibiting greater leaf length in July ($P=0.0401$, $F_{1,131}=4.30$, $r^2=0.03$). The Oregon seed source produced the greatest total leaf length in July, followed by the Idaho, local, and California sources respectively (Figure 5).

DISCUSSION

With significant source level differences detected across all performance measurements, seed source appears to be an important component of seedling performance in big squirreltail. Emergence was strongly affected by seed source, with the local source emerging significantly earlier than the other sources and producing the greatest quantity of emerged seedlings (Table 1). Likewise, seedling active growth was closely tied to seed source throughout the growing season (Table 1). Local seed proved the superior performer from March through June and remained among the top two for July and January (Figure 4). Only in September did the local source exhibit relatively poor active growth. The fact that the largest portion of surviving plants were from the local source at the conclusion of first year monitoring suggests that local seed will remain a strong performer after dormancy. Follow-up monitoring of second and third year seedling performance might provide further insight into the relationship of seed source and fitness.

Seedling performance can be affected by both genetic factors and inherited non-genetic factors, in particular maternal effects (Roach and Wulff 1986). Seed weight can be influenced through non-genetic maternal provisioning (Eagles and Hardacre 1979; Antonovics and Schmitt 1986). Seed weight often correlates with initial seedling performance (Wulff 1986; Kitajima 2007), making the possibility of maternal control relevant to our finding of performance differences between seed sources, although many other potential types of maternal effects exist. We observed differences in seed size between source populations, but we could not determine whether that variation is under maternal or genetic control. The impact of maternal effects can be confirmed by

comparing traits between field-collected seed and seed collected in a common garden (Mazer 1995; Roach and Wulff 1987). Our common garden plants have yet to produce seed for comparison.

Even if seed weight was maternally-influenced, its effects were limited. We observed a very tight range of seed weights with substantial overlap in variance between seed sources (Figure 1); given a seed weight, identification of its source population would be difficult. The only attribute significantly impacted by seed weight was emergence percentage, with bigger seeds being more likely to emerge. Seeds from local sources, which exhibited the second lowest mean seed weight (Figure 1), still produced the greatest quantity of both emergent and surviving plants (Figure 4). In this experiment, seed weight was a poor indicator of seedling performance.

When seeds were competing with extant field plants, they were less likely to emerge and had later emergence dates (Table 1). Competition from existing vegetation generally reduces seedling survivorship (Fenner 1978; Aguilera and Laurenroth 1993; Tyler and D'Antonio 1995). However, any depressive effect from competition was probably limited to the establishment phase. Competition status at sowing did not have a strong influence on survivorship; it was not correlated to active growth in any of the six growth assessments (Table 1b).

Plants that emerged earlier exhibited a significantly higher percentage of active growth across the entirety of the growing season as well as higher mean leaf lengths at the end of the first growing season. Early emergence has been shown to increase both short-term survivorship and long-term (9-year) fitness in perennials (Verdu and Traveset 2005; DeLuis et al 2008). Seedlings that germinate first can become competitive

dominants through space and resource preemption (Ross and Harper 1972; Dyer et al. 2000). Similarly, perennials that initiate growth early in the season may be able to preempt the colonization of annual species (James et al. 2006). The strong correlation between early emergence and first season survivorship may warrant further investigation into the use of emergence timing as a screening tool for future *Elymus multisetus* seed intended for use as revegetation material.

At the end of the first growing season, both mean and total leaf length was affected by seed source, with large differences in total leaf length between sources. Leaf length of *E. multisetus* is highly correlated with plant biomass (Leger 2008), and plant biomass is often correlated with fecundity and competitive ability (Scheiner 1987; Aarssen 1992; Keddy et al. 2002). Local plants produced the lowest mean leaf length and the second lowest total leaf length (Figure 5). In terms of mean leaf length, local seedlings were vastly outperformed by both the Idaho and Oregon sources. Since an equal amount of seed was sown for each source in this experiment, higher total leaf length amounts to a greater biomass return for equal seed investment. For restorationists with a goal of maximizing cover from native vegetation, biomass return on seed input is perhaps a more important success criterion than mean leaf length or survivorship percentage. Local seeds produced a mean leaf length that averaged below the grand mean; however, when total length production is summed across sources, local material accounted for 25% of all leaf length—the expected mean value—because it had the greatest survivorship percentage. Idaho and Oregon sources still produced much more biomass, but increased survival of the local seed may compensate for its low mean leaf length.

Nonetheless, if size is a reliable indicator of fitness, then the relatively small size of local plants may hinder their long-term fitness, despite the greater emergence and survivorship of local seedlings. However, there are circumstances in which being small could actually be adaptive (Aarssen et al. 2006). In highly competitive environments, fecundity is not always closely correlated to biomass (Chambers and Aarssen 2009). Small plants can contribute disproportionately to seed production (Neytcheva and Aarssen 2007). Conversely, there can be competitive disadvantages to greater biomass, especially in drought conditions, where large plants generally experience greater transpiration losses (Tyree 2007).

The superior performance of local seed that we observed is consistent with local adaptation. In both emergence and first season survivorship, local seed clearly outperformed all regional sources. While local seedlings produced relatively low biomass, environmental plasticity in biomass is widely documented and may be decoupled from fitness (Bradshaw 1965; Schlichting 1986). Phenological traits, such as emergence timing, are often closely correlated with seed source environmental conditions, lending support to the idea that these are adaptive traits (Meyer and Monson 1991, Meyer 1992; Erickson et al. 2004). For perennial species, the quantification of lifetime fitness requires measurement of survivorship and fecundity over several years and is rarely attained in anything but long-term ecological monitoring. Although short-term performance measures such as emergence and seedling survivorship do not capture lifetime fitness, they can be fast and efficient methods to experimentally differentiate between populations under consideration for use in revegetation. More refined testing,

especially reciprocal transplant experiments, is necessary before a more definitive conclusion about the scale of local adaptation in the Great Basin can be reached.

CONCLUSION

Our experiment represents a first step in evaluating the role of seed source in post-fire revegetation seedling establishment in the Great Basin. Almost all response variables we measured were affected by seed source, including emergence percentage and timing, first season survivorship, and leaf length. The differential seedling establishment success exhibited across seed sources suggests that there may be fitness differences between *Elymus multisetus* populations under post-fire revegetation conditions. Certain populations may possess adaptive traits that allow for enhanced performance under these conditions. The decision to introduce regionally farm-produced big squirreltail seed at revegetation sites in the Great Basin should be accompanied by either review or field evaluation of the performance of seed material under conditions similar to proposed revegetation. In the absence of clear evidence that commercially-available seed material exhibits superior performance at a given site, restorationists should consider collecting and revegetating with local seed. The introduction of farm-grown seed may significantly impact both restoration success and local population genetic variation.

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TABLES

Table 1. Results of Chi-square test and analysis of variance testing the effects of seed source, seed weight, competition status on emergence, July leaf length, and active growth. Bolded results are significant ($\alpha=0.05$).

	a.	n	R ²	Source			Seed weight			Competition at sowing		
				df	F	P > F	df	F	P > F	df	F	P > F
Days to Emergence		557	0.09	3	16.2	<0.0001	1	3.3	0.0689	1	10	0.0015
July Mean Leaf Length		131	0.07	2	4.68	0.011	1	0	0.976	1	0.41	0.5241
Emergence Percentage	b.	n	R ²	df	χ^2	P > χ^2	df	χ^2	P > χ^2	df	χ^2	P > χ^2
		1000	0.29	3	374.38	<0.0001	1	11.74	0.0006	1	3.41	0.0646
					Active Growth							
April		1000	0.28	3	365.39	<0.0001	1	13.46	0.0002	1	0.57	0.4491
May		1000	0.26	3	338.45	<0.0001	1	11.68	0.0006	1	0.12	0.7266
June		1000	0.13	3	123.98	<0.0001	1	5.99	0.0144	1	2.79	0.0951
July		1000	0.11	3	85.76	<0.0001	1	8.76	0.0031	1	0.74	0.3882
September		1000	0.08	3	16.88	0.0007	1	2.17	0.1411	1	0.97	0.3253
January		1000	0.07	3	23.25	<0.0001	1	2.4	0.121	1	0.11	0.7323

FIGURE LEGENDS

Figure 1. Distribution of seed weights by seed source. Center line of box plots represents median seed weights for each seed source, box outline represents $\pm 25\%$ from the median and bars represent 10% and 90% quartiles. Letters indicate significant ($\alpha=0.05$) differences between means.

Figure 2. Differences in emergence percentage among seed sources. Percentages have not been adjusted by viability. Grey hatched areas represents percentage of seeds that did not emerge.

Figure 3. Days to emergence by seed source (mean value \pm SE). Mean emergence dates are indicated. Significant differences ($\alpha=0.05$) among seed sources are indicated with lowercase letters.

Figure 4. Plants exhibiting active growth (a proxy for survivorship) by seed source by month. Values have not been adjusted by viability. Measurements were taken in March, April, June, July, and November 2009 and January 2010. Asterisks indicate significant differences ($\alpha=0.05$) between seed sources.

Figure 5. Total leaf length by seed source in July. Values represent the summation of all leaf lengths of all plants exhibiting active growth at the end of the first growing season and therefore do not have error terms.

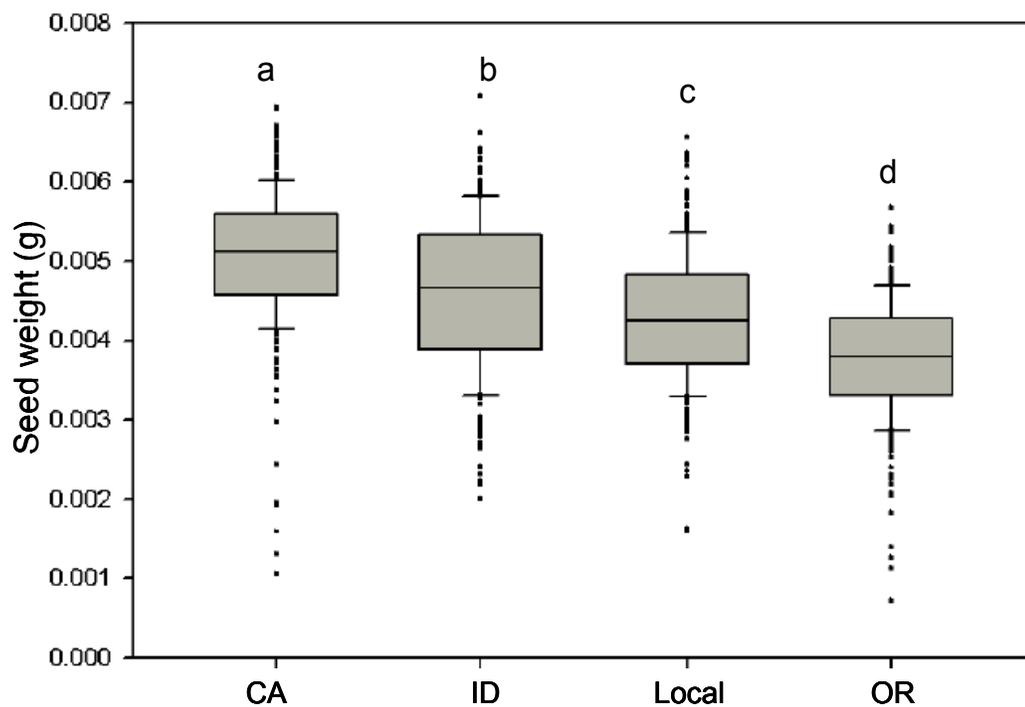
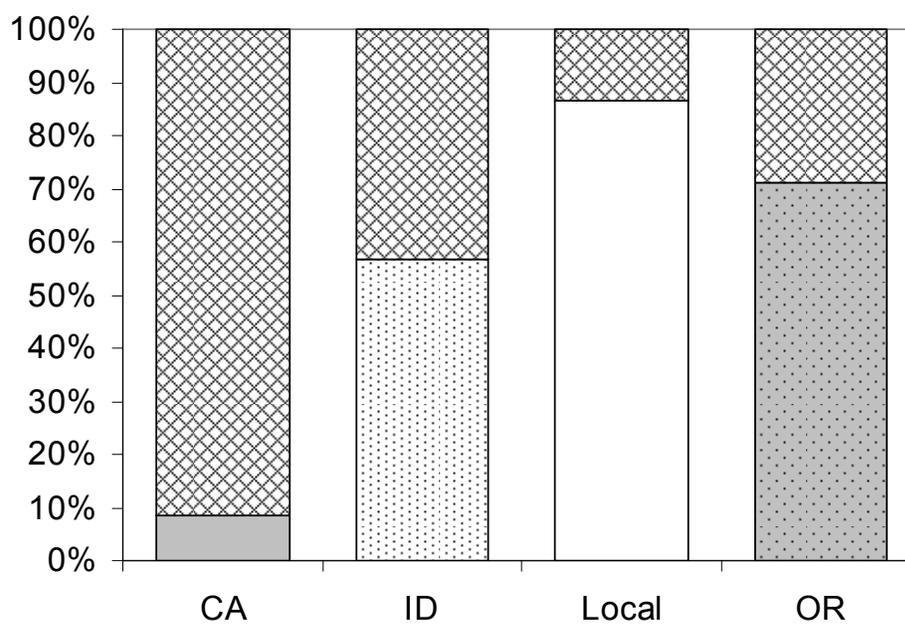
FIGURES**Figure 1****Figure 2**

Figure 3

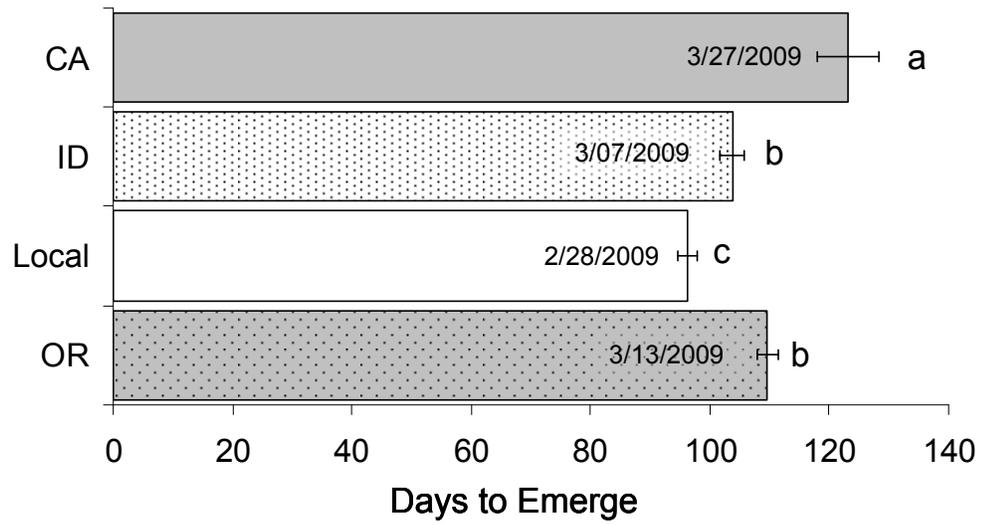


Figure 4

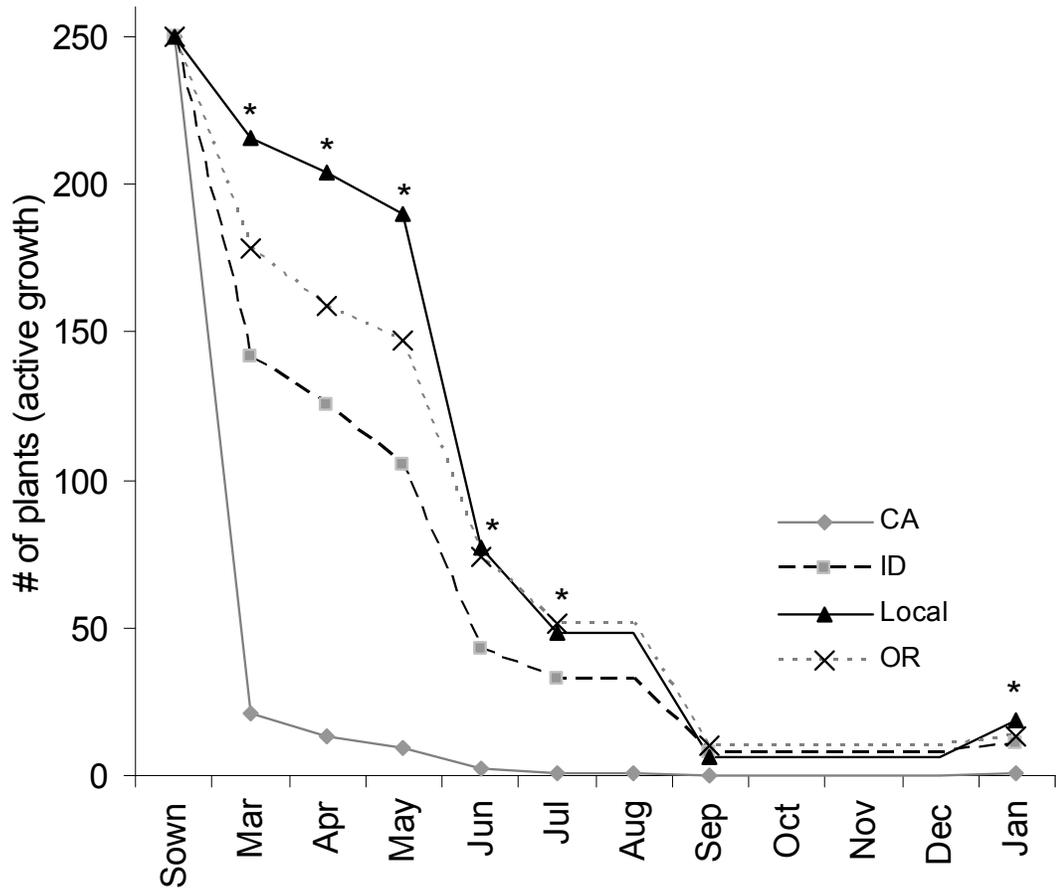
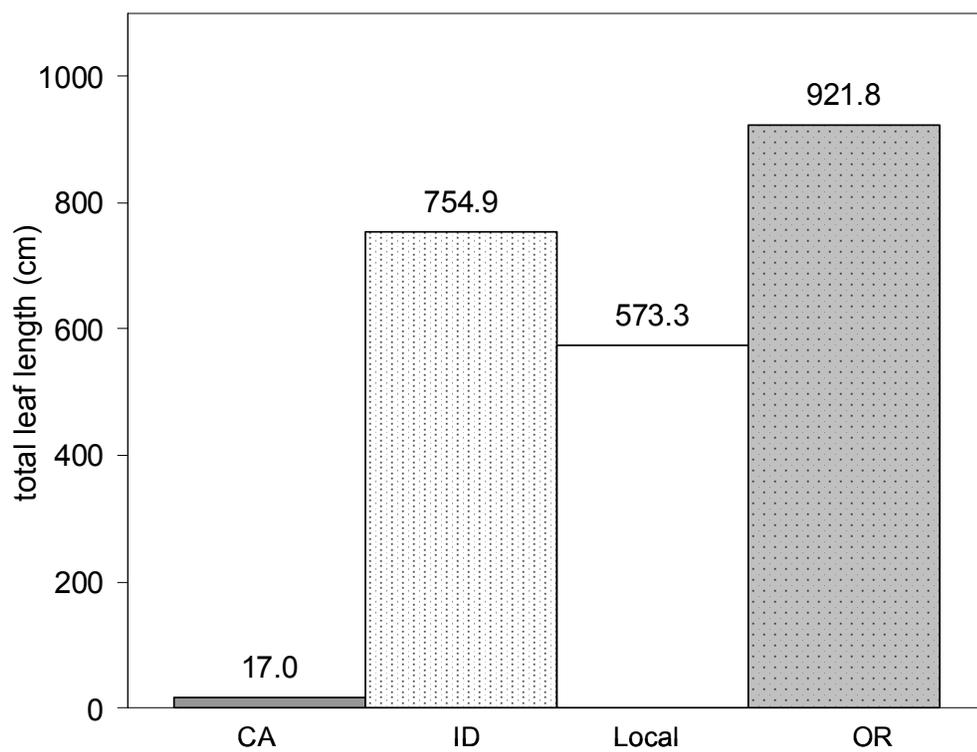


Figure 5



Competitive seedlings and inherited traits: a test of rapid evolution of *Elymus multisetus* (big squirreltail) in response to cheatgrass invasion

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ABSTRACT

Widespread invasion by *Bromus tectorum* (cheatgrass) has drastically altered native plant populations. We investigated whether *Elymus multisetus* (big squirreltail) is evolving in response to invasion and what traits may contribute to competitive ability. Seedlings from invaded areas exhibited greater tolerance of competition and a greater ability to suppress cheatgrass than seedlings from adjacent uninvaded areas. Root and shoot growth traits at 10, 50 and 100 days were obtained through destructive harvesting. To identify adaptive traits, we examined which traits were correlated with competitive ability within the presumed ancestral gene pool, determined their genetic variation by measuring sibling resemblance, and asked if these traits had shifted in cheatgrass-invaded areas. Competitive ability was correlated with 10 day root:shoot, root forks, and fine root length, but only 10 day root forks appear to be inherited and none have significantly shifted across invasion status. Additionally, we surveyed traits that varied between invaded and uninvaded sources. Invaded plants were smaller, allocated more biomass to roots, and produced a higher percentage of fine roots. Root traits, including early root growth, may be an important component of competitive ability in *E. multisetus*. The ability of native populations to evolve in response to invasion has significant implications for management and restoration of cheatgrass-invaded communities.

KEYWORDS

Bromus tectorum; cheatgrass; *Elymus multisetus*; big squirreltail; competition; invasive species; local adaptation; natural selection; rapid evolution; restoration

INTRODUCTION

The impact of invasive species on native species, communities, and ecosystem processes has been recognized for decades (Elton 1958; Lodge 1993; Wilcove et al. 1998). Biological invasions dramatically affect the distribution, abundance, and reproduction of many native species (Williamson 1996, Parker et al. 1999; Mack et al. 2000). While biological invasions are considered a significant threat to biodiversity (Sala et al. 2000; Stein et al. 2000), they do not result in the extinction of the majority of native species impacted; many native species are able to persist alongside invaders (Hironaka and Tisdale 1963; Morrison et al. 2002; Levine and Rees 2004; Meador et al. 2004; Stohlgren et al. 2006). These persistent native species may possess traits (i.e. preadaptations) or sufficient trait plasticity that allow for successful coexistence with invaders (Stearns 1989; MacNeil 2001; Carroll et al. 2005). Preadapted traits can increase in frequency in invaded populations, or under the right circumstances, native species may evolve new mechanisms, such as chemical defenses or improved competitive ability, to cope with the invaders. Both types of rapid evolutionary responses have been documented in native populations (Schlaepfer et al. 2005; Strauss et al. 2006). Most research involving rapid evolution in natives responding to invaders has focused on native-invader interactions across trophic levels (e.g. predator-prey, host-pathogen relationships, Strauss et al. 2006). To date, there are few studies that address evolutionary response of native plant populations to plant invaders (e.g. Callaway et al. 2005; Lau 2006; Meador and Hild 2007; Leger 2008; Cipollini 2009).

The conversion of Great Basin rangeland to *Bromus tectorum* L. (cheatgrass) is one of the most severe ecological degradations in the United States (Mack 1981;

D'Antonio and Vitousek 1992). The combination of heavy grazing, which reduces the vigor of native perennial species, and cheatgrass invasion, which shortens fire return interval and alters nutrient cycling, has resulted in large-scale conversion of *Artemisia* spp. (sagebrush)-dominated communities to cheatgrass-dominated plant communities (Pechanec and Hull 1945; Young and Evans 1972; Evans et al. 2001; Brooks et al. 2004; Chambers et al. 2007). By 2000, nearly 12.7 million hectares of the Great Basin had been converted to cheatgrass and 45% was estimated to be at risk of conversion (Menakis et al. 2003; Bradley and Mustard 2005). Control and restoration attempts in cheatgrass-invaded areas are now common management practices (BLM 2001), but the scale of invasion makes the extirpation of cheatgrass unlikely (Young 2000).

Native perennial grasses in the Great Basin have experienced a shift in composition of interspecific competitors from native perennial species to cheatgrass. Cheatgrass has strong negative impacts on native perennial plant fitness (Rummell 1946; Aguirre and Johnson 1991; Nasri and Doescher 1995; Rafferty and Young 2002). A few native grass species appear to be relatively tolerant of cheatgrass invasion, including *Elymus multisetus* M.E. Jones (big squirreltail) and *E. elymoides* Raf. (Swezey) (bottlebrush squirreltail) (Hironaka and Tisdale 1963; Humphrey and Schupp 1999; Booth et al. 2003; Leger 2008). These two species are so closely related that a subspecific ranking of *E. multisetus* is debated (Holmgren and Holmgren 1978; Barkworth et al. 1983; Arnow 1993; Jones et al. 2003; Larson et al. 2003). Both are highly selfing, short-lived perennials that reproduce effectively from seed, persist in disturbed areas and survive moderate fires (Young and Larson 1977; Dewey 1988; Britton 1990; Jones et al. 1998). A tolerance for disturbance has made both squirreltail

species of interest for restoration of burned and cheatgrass-invaded areas (Hardagree 1994; Richards 1998; Humphrey and Schupp 2002). Two commercial germplasm releases of *Elymus elymoides* and one release of *E. multisetus* have been developed for use in restoration in the Great Basin (Jones et al. 1998, 2004a,b). The ability of intact squirreltail populations to resist invasion may result from lower susceptibility of mature plants to competitive suppression rather than from seedling competitive ability (Humphrey and Schupp 2004). Direct comparison between squirreltail and annual grass competitors in relative growth rate (Hironaka 1975; Arredondo et al. 1998; Young and Mangold 2008), germination percentage and phenology (Young et al. 2003), and nutrient use efficiency (Redente et al. 1992) usually favor the invader, suggesting that no individual strategy can account for squirreltail's tolerance of invasion.

Increased ability to tolerate cheatgrass invasion has been demonstrated in mature *Elymus multisetus* plants collected from cheatgrass invaded areas, but shifts in seedling performance have not been investigated (Leger 2008). The transition from seed to seedling is the most vulnerable stage of plant life (Kitajima 2007), so examining seedling performance is important for understanding early survivorship which is a large component of fitness. Phenotypic variation, heritability of traits and fitness differences between phenotypes are required for natural selection. Any trait that increases seedling establishment in cheatgrass invaded areas would likely be favored by natural selection. Performance of seeds from the same plant (i.e. siblings) can provide a tractable way to quantify genetic variation and estimate heritability. Traits that exhibit significantly greater similarity among plants from the same parent (i.e. sibling resemblance) when compared to other plants within the same population are assumed to be inherited

(Gurevitch et al. 2002). Family level correlations have been used for decades to address questions of inheritance and natural selection (Endler 1978; Falconer and McKay 1996; Roff 1997), but are not frequently applied in the context of native plant restoration.

The goals of this study are two fold: 1) to test whether the differential competitive ability found in mature *Elymus multisetus* plants from invaded areas (Leger 2008) is inherited in seeds collected from the same area; 2) to identify what growth traits may help *Elymus multisetus* to establish or persist in cheatgrass-invaded areas and whether those traits exhibit genetic variation. We conducted a common garden greenhouse experiment with maternal families from invaded and uninvaded areas. We identified growth traits that were correlated with increased tolerance of cheatgrass competition, measured the genetic variation of these traits, and asked if they have shifted between uninvaded and invaded cohorts. Next, we surveyed all growth traits that varied between uninvaded and invaded cohorts to identify additional traits that may confer competitive advantage to native plants growing in invaded areas.

MATERIALS AND METHODS

Seed Collection Methods

Seeds of *Elymus multisetus* were collected in June 2007 from Balls Canyon, Sierra County, California (39840.740 N, 120803.360 W, 1600 m elevation), where a shift in invasion status occurs over a relatively small geographic area (ranging from 0% cheatgrass in uninvaded areas to 40% cheatgrass cover in invaded areas). Invaded and uninvaded areas occur in very close proximity; all collection sites were within 1.2km of each other. Detailed description and community composition of collection sites are

recorded in Leger (2008). Seeds were collected from up to 40 individuals from four invaded and uninvaded collection sites and stored at room temperature. Seeds collected from a single plant were labeled as a separate family. Twenty-five families were randomly selected from cheatgrass invaded sites and uninvaded areas (50 families total). Depending on seed availability, 18-20 seeds from each family were used for this experiment (991 plants total). Families and seedlings from invaded collection sites are subsequently referred to as 'invaded families' and 'invaded plants,' and families and seedlings from uninvaded collection sites, 'uninvaded families' and 'uninvaded plants.'

Greenhouse and Data Collection Methods

The experiment was conducted under controlled greenhouse conditions: 4.4-26.6°C temperature range; 5-25% relative humidity; full daytime sunlight. Potting mix was locally produced (R.C. Donovan, Reno, NV) and contained bark, compost, decomposed granite, and perlite. Laboratory tests of the potting mix revealed very low mineral N (3 ppm), high alkalinity (pH=8.0), and low estimated water holding capacity (44%) (A and L Western Agricultural Laboratories Report #08-136-060 2008). To allow for maximum rooting depth, we used pots designed for revegetation horticulture (Stuewe and Sons TPOT1; 10.2 cm x 10.2 cm x 35.6 cm; 3.2L). We pre-watered pots for two days prior to sowing. Seeds received no pregermination treatment and were directly sown into pots on 26 March 2008. In each pot, we sowed one individually weighed *Elymus multisetus* seed and placed a toothpick adjacent to seed for germination identification. To create competitive conditions, we sowed five cheatgrass seeds into each of 14-16 pots for each squirreltail family (n=791). An additional four pots per family were established as non-competing controls (n=200). Pots for the same family

were sown together and then moved into random greenhouse positioning to achieve a complete random design. We misted seeds immediately after sowing and continued misting twice daily for two weeks. For the remainder of the experiment, plants were hand-watered to saturation when the bottom third of root zone was dry (4-14 day intervals) and no supplemental fertilizer was added.

We measured or calculated a total of 47 growth traits: seed weight; emergence date; early growth rate (leaf number and length); root, shoot, and total biomass weight (mg) at 10, 50, and 100 days; leaf number and root:shoot ratio (R:S) at 10, 50, and 100 days; total root length (cm), root diameter (mm), number of root tips, number of root forks, and root length in five 0.1mm size classes at 10, 50, and 100 days.

Three sequential harvests were conducted on seedlings grown in competition with cheatgrass. Harvest dates were selected to correspond to important phenological benchmarks, including seedling establishment phase (10 days postgermination), the active growth phase (50 days), and the end of growing season (100 days). Plants for harvest were randomly selected within families. Two plants per family (n=100) were harvested at 10 days (14-18 April 2008), and three plants per family (n=150) were harvested at 50 days (16-18 May 2008). At 100 days, due to the time requirements for root harvest, we were only able to harvest one competing and one non-competing plant per family (n=100) (15 July 2008). At 100 days, leaf number was recorded for all live plants (n=669).

To extract roots at harvest, we cut away pots and gently removed planting media by misting with water. *Elymus multisetus* were identified by leaf and root characteristics and manually separated from cheatgrass roots. Very little root breakage occurred;

nonetheless, we noted when roots were broken during extraction, so that these plants could be excluded from analysis, if necessary. Harvested roots and shoots were refrigerated (<48 hours), then digitally scanned for analysis.

Emergence date was recorded daily at 7am from sowing date until last recorded emergence on 15 April 2008. For a subset of 406 seedlings, leaf length and number were measured on 23 April and 15 May 2008. Early seedling growth rate was quantified as $[\text{May leaf number} - \text{April leaf number}] \div \text{May leaf number}$. Root and shoot biomass measurements were obtained by drying (7 days at 60°C) and weighing root and shoot material separately. Total biomass was calculated as shoot + root weight. Root:shoot ratio (R:S) was calculated as $\text{root weight} \div \text{shoot weight}$. WinRhizo root-scanning software (Regent Instruments Inc, Saint-Foy, Canada) was utilized to quantify the following root characteristics—total root length (cm), root diameter (mm), number of root tips, number of root forks, and root length (cm) in five 0.1mm size classes (user-defined). Root forks and tips are measurements of root branching. To analyze genetic control of seed weight, next-generation seed was collected and weighted from a subset of non-competing control plants that remained after harvesting.

Relative competitive performance index (CPI) was used to quantify seedling tolerance to cheatgrass competitors—our primary measurement of competitive ability (Keddy et al 1998). CPI is the percent decrease in plant performance when grown with competitors and was calculated as: $[\text{Leaf number without competition} - \text{leaf number with competition}] \div \text{leaf number without competition}$. Leaf number is strongly correlated with biomass in *Elymus multisetus* (Leger 2008) and was used as a proxy for biomass in CPI calculation. Because individual seedlings could not be exposed to both competition and

control conditions, we used family means for leaf number with and without competition to estimate biomass, and these values were used for CPI calculation. To quantify cheatgrass biomass suppression, cheatgrass shoot biomass was collected from all pots containing cheatgrass (n=507) at 100 days. In 32 competition pots, there was no *E. multisetus* emergent, but 4 or more cheatgrass emergents. These pots were used to calculate mean cheatgrass weight grown alone. Cheatgrass shoots were dried and weighed in same manner as *E. multisetus*.

Analysis Methods

Statistical analyses were performed using JMP 7.0.2 (SAS Institute 2007). Analysis of variance (ANOVA) was used to compare the effects of invasion status and family on 47 growth traits of *Elymus multisetus* seedlings in competition. For seed weight, emergence date, early growth rates, 10 day harvest data, 50 day harvest data, 100 day leaf number, and next generation seed weight, the ANOVA model included the following factors: invasion status (invaded or uninvaded); collection site (random factor, nested in status); family (random factor, nested in status and site). Family was removed from ANOVA models for the analysis of 100 day biomass and 100 day root traits, because only one plant per family was measured in each competition condition, as well as from ANOVA of CPI, because they were calculated using family means. Five outlier data points were excluded from 100 day leaf number and CPI analysis because leaf number exceeded three standard deviations from the mean; however, these points were included in early trait analysis, where values fell within the range established by other family data points of the same family. Seed weight and emergence date comparisons included all data, rather than just competing plants, because these traits were not affected

by competition condition. For cheatgrass biomass, two separate ANOVAs were conducted to analyze the effect of competition condition (cheatgrass grown alone, grown with invaded *Elymus multisetus*, or grown with uninvaded *E. multisetus*) and family of *E. multisetus* competitor. For this second analysis of cheatgrass biomass, *E. multisetus* family was nested within collection site. When analyses were significant, means were compared *a posteriori* using Tukey HSD. Several growth traits did not display normal distributions; however, transformation did not affect detection of significance, so raw values were used. Raw means, rather than least means square, are reported for all growth trait and competitive ability measurements. Spearman's ρ was used for non-parametric multivariate analysis of family mean CPI and family means for each growth trait among uninvaded families. No adjustments were made for multiple comparisons because we wanted to maximize the chances of finding potentially important traits, and the time-intensive nature of these measurements constrained sample sizes. Pairwise linear regression was conducted for traits that were significantly correlated with CPI in non-parametric analysis ($\alpha=0.05$).

RESULTS

At 100 days, *Elymus multisetus* plants grown in competition with cheatgrass had 81.4% less total biomass ($P<0.0001$, $F_{1,100}=46.88$, $r^2=0.32$), 70% less root biomass ($P<0.0001$, $F_{1,100}=33.15$, $r^2=0.25$) and 90.2% less shoot biomass ($P<0.0001$, $F_{1,101}=51.80$, $r^2=0.34$) than control plants grown without competition. Leaf number decreased by 82.5% with competition ($P<0.0001$, $F_{1,672}=708.12$, $r^2=0.51$). At 100 days, leaf number was highly correlated with total biomass ($P<0.0001$, $F_{1,100}=672.98$, $r^2=0.87$), root biomass

($P < 0.0001$, $F_{1,100} = 223.42$, $r^2 = 0.70$), and shoot biomass ($P < 0.0001$, $F_{1,101} = 10005.48$, $r^2 = 0.91$). Due to a relatively large sample size ($n = 669$), leaf number exhibited very high power to detect differences between plants of different invasion statuses and families, for both competing plants ($\text{power}_{\text{status}} = 1.0000$; $\text{power}_{\text{family}} = 1.0000$) as well as non-competing control plants ($\text{power}_{\text{status}} = 1.0000$; $\text{power}_{\text{family}} = 0.9987$). Leaf number was, therefore, an appropriate proxy for total biomass in the calculation of a competitive performance index at 100 days. Fifty day leaf number was also highly correlated with 50 day total, root, and shoot biomass (all $P < 0.001$). However, at 10 days, leaf number was not significantly correlated with any biomass measure (all $P > 0.05$), as most plants had ≤ 3 leaves at this time, regardless of their size.

Competitive Performance Index and Cheatgrass Suppression

Competitive performance index (CPI) for leaf number varied significantly by invasion status ($P = 0.0160$, $F_{1,50} = 6.30$, $r^2 = 0.31$) and collection site ($P = 0.0496$, $F_{6,50} = 2.33$, $r^2 = 0.31$). Mean CPI of invaded families was significantly lower ($77.0\% \pm 1.1\%$) than that of uninvaded families ($80.2\% \pm 1.0\%$) (Figure 1). Of the top ten cheatgrass tolerating families, eight were from invaded areas. Cheatgrass shoot biomass varied significantly between control plants (i.e. grown alone), those grown with invaded status *E. multisetus*, and those grown with uninvaded status *E. multisetus* ($P < 0.0001$, $F_{2,508} = 60.55$, $r^2 = 0.19$) (Figure 2). Cheatgrass shoot biomass was highest for cheatgrass plants grown with uninvaded *E. multisetus* ($2.30 \pm 0.10\text{g}$), followed by cheatgrass alone ($1.59 \pm 0.21\text{g}$), and cheatgrass growth with invaded *E. multisetus* ($1.03 \pm 0.05\text{g}$). Cheatgrass weight also varied significantly among the *E. multisetus* families with which it was grown ($P < 0.0001$, $F_{42,476} = 5.73$, $r^2 = 0.68$). Additionally, ranking of cheatgrass biomass by family

revealed that seven of the ten families with the lowest cheatgrass biomass (i.e. most suppressive) were from invaded areas (Figure 3).

Growth Traits in a Competitive Environment

When grown in competition with cheatgrass, *Elymus multisetus* seedlings exhibited 15 growth traits that varied significantly by invasion status and 17 growth traits that varied significantly by family (Table 1). Of the 22 growth traits that varied significantly by invasion status or family, 59% (13 traits) were found in the 50 day harvest group when we had increased statistical power due to greater sample size, while none were from the 10 day harvest group.

Plants from invaded areas exhibited significantly lower seed weights (4.32 ± 0.046 mg) than uninvaded plants (4.78 ± 0.039 mg). Similarly, in early growth of both leaf number and leaf length, invaded plants exhibited significantly slower growth rates (0.33 ± 0.013 leaves gained; 0.26 ± 0.013 cm gained) than uninvaded plants (0.45 ± 0.018 leaves gained; 0.39 ± 0.035 cm gained, Table 1). At each harvest date, invaded plants exhibited lower root, shoot, and total biomass, with significant differences in shoot and total biomass at 50 days and in all three measures at 100 days (Table 1; Figure 4). Invaded plants exhibited higher R:S at 50 and 100 days, but not at 10 days; this difference was significant at 50 days (Table 1; Figure 4). At both 50 and 100 days, invaded plants exhibited significantly lower leaf numbers than uninvaded plants (Table 1; Table 2). The average root diameter of invaded plants at 50 days was significantly smaller than that of uninvaded plants (Table 1; Table 2). The total length of large diameter roots (>0.400 mm) varied significantly by invasion status, with uninvaded plants exhibiting greater length of large diameter roots than invaded plants (Table 1;

Table 2). Finally, at each harvest date, invaded status seedlings exhibited higher percentages of fine roots than uninvaded seedlings, with significant differences at 50 days (Table 1; Table 2).

Three root diameter size classes exhibited detectable family level differences at 50 days (Table 1; Figure 5). There were no family level differences in root distribution detected at 10 or 100 days (all $P > 0.05$). Across both family and invasion status level differences, there was a marked temporal difference in root growth patterns between 10 and 50 days (Figure 5). At 10 days, there were very few roots in the smallest size class ($< 0.100\text{mm}$), with the greatest root lengths occurring between $0.101\text{-}0.200\text{mm}$. By 50 days, there was a proliferation of roots in the smallest size class, and total root lengths decreased with increasing size class. At 100 days, size class distribution exhibits a similar pattern to 50 days (results not shown). Examination of photographic root scans at 10 and 50 days confirmed the temporal shift in development of roots in different size classes (photos not shown). In general, roots $< 0.100\text{mm}$ in diameter were absent at 10 days, but present by 50 days.

Within uninvaded status families, there were three growth traits that correlated significantly with CPI: 10 day R:S ($P = 0.0178$), 10 day root forks ($P = 0.0427$), and 10 day length of small diameter roots ($0.101\text{-}0.200\text{mm}$) ($P = 0.0309$). Non-significant findings were not reported ($\alpha = 0.05$). Lower CPI (i.e. greater competitive ability) was correlated with higher 10 day R:S, more root forks, and greater total length of small diameter roots (Figure 6). While 10 day root forks did not vary significantly between families when considering plants from both invaded and uninvaded areas (Table 1), when looking only among uninvaded plants, 10 day root forks varied significantly by family ($P = 0.0224$,

$F_{21,49}=2.13$, $r^2=0.70$). Neither 10 day R:S nor the length of small diameter roots varied at between families within uninvaded plants. Analysis of all harvested plants did not detect differences between invaded and uninvaded cohorts for 10 day root forks, R:S, or small diameter (0.101-0.200mm) roots. For these three traits, power analysis revealed large sample size requirements (>1000) to detect invasion status differences.

Seed weight

Several early growth traits were significantly correlated with seed weight. Ten day root biomass ($P<0.0001$, $F_{2,99}=28.13$, $r^2=0.22$), shoot biomass ($P<0.0001$, $F_{2,100}=26.62$, $r^2=0.22$), and total biomass ($P<0.0001$, $F_{2,99}=36.10$, $r^2=0.27$) increased with increasing seed weight. The length of roots in the three largest diameter classes root classes also increased with increasing seed weight (all $P<0.02$). No 50 day or 100 day growth traits correlated with seed weight (all $P>0.05$). Neither CPI nor cheatgrass biomass varied by seed weight ($P>0.05$). Next generation seed weights (i.e. collected from non-competing control plants grown in common garden) varied significantly by invasion status ($P<0.0001$, $F_{1,200}=209.72$, $r^2=0.91$), with invaded plants producing smaller seeds ($5.2\pm 0.04\text{mg}$) than uninvaded plants ($6.1\pm 0.04\text{mg}$). Next generation seed weight also varied significantly among families ($P<0.0001$, $F_{1,200}=33.10$, $r^2=0.91$).

DISCUSSION

The invasion of cheatgrass is converting large areas of the Great Basin from native perennial-dominated to cheatgrass-dominated plant communities. Some native plants are able to persist in these invaded areas, and may evolve in response to long-term cheatgrass presence. We found that *Elymus multisetus* seedlings from invaded areas were

more tolerant of competition and better able to suppress cheatgrass than plants from nearby uninvaded areas. Although no growth traits exhibiting genetic variation could be definitively correlated with the shift in competitive ability observed across invasion status, several important growth traits differed between invaded and uninvaded cohorts.

Regardless of seed source, all plants grown in competition with cheatgrass were smaller, with lower mean leaf numbers across all families for both invaded and uninvaded cohorts, indicating competitive suppression from cheatgrass. This effect allowed for the calculation of a competitive performance index and was foundational for the hypothesis that cheatgrass is a strong selection agent. Since CPI is a measure of ability to tolerate competition in our experiment, then traits that are associated with lower CPI may contribute to improved performance in a cheatgrass-competitive environment and may be considered adaptive.

Invaded plants exhibited significantly lower CPI (i.e. less biomass loss when in competition) (Figure 1), indicating that they were better tolerators of cheatgrass competition than their uninvaded cohorts. The difference was small—a 3% decrease in CPI for invaded plants. Nonetheless, invaded plants were also better able to suppress cheatgrass biomass compared to their uninvaded cohorts (Figure 2). The ability to suppress cheatgrass biomass varied significantly by family, indicating genetic variation and possibly heritability (Figure 3). Additional field testing is necessary to determine if the lower CPI and greater ability to suppress cheatgrass exhibited by invaded seedlings will result in higher lifetime fitness for these plants in invaded areas. Nonetheless, the fact that plants from adjacent seed sources exhibit differential ability to both tolerate and suppress cheatgrass based upon their previous exposure to cheatgrass, and that this ability

is inherited, is consistent with the hypothesis that cheatgrass is exerting a selection pressure.

Our most conservative approach for examining adaptive traits was a three-step process of assessing which traits are correlated with CPI within the presumed gene pool (i.e. uninvaded plants), determining their level of genetic variation, and examining if trait values have shifted in the invaded conspecifics. If populations are evolving in response to cheatgrass pressure, then there should be a shift in inherited CPI-correlated traits across invasion status. Of the 47 growth traits measured, there were only three traits that correlated to CPI within uninvaded families, all of which were related to root growth at 10 days. We did not detect a shift across invasion status for these traits, possibly due to low power. Nonetheless, we believe that this methodology is appropriate for verifying not only what traits may be important for cheatgrass competition, but also that those traits can evolve in natural populations. Identifying traits that are easier to measure and are reasonable proxies for root traits (e.g. resource capture) may be a way to improve the resolution of this method.

Better competitive performance was associated with greater fine root production, higher R:S and more root forking (Figure 6). This would suggest that early root growth plays a significant role in competitive ability for *Elymus multisetus* seedlings. In nutrient poor environments, allocation to below-ground resource capture (e.g. high R:S, high specific root length, high fine root production) improves competitive ability (Tilman 1988; Aerts and Chapin 2000). Among six Great Basin species, the ability to produce fine roots in response to nutrient pulses was correlated with relative growth rate and conferred competitive advantage (Bilbrough and Caldwell 1997). Likewise, increased

root biomass and nitrogen uptake rate allowed invasive forbs to capture 2- to 15-fold more nitrogen than several Great Basin grasses and forbs in short-lived high-nutrient patches (James 2009).

The relevance of root forks to competitive ability is more ambiguous, as the importance of lateral root branching for resource capture is still relatively unexplored (Lynch 1995). In nutrient poor-environments, root morphology rather than physiology may confer the greatest advantage in nutrient uptake ability (Aerts 1999). In an examination of genetic variation of root morphology in white clover, it was suggested that genotypes with a high number of root tips and forks will possess highly branched root systems, which explore a large volume of soil per unit root weight, thereby improving nutrient uptake (Jahufer et al. 2008). When responding to high-nutrient patches, the roots of 11 herbaceous species had a more herringbone branching patterns than roots in low-nutrient patches (Fitter 1994). Soil nutrient heterogeneity was not a component of our study. It is, however, an important factor in our study system, where nutrients are believed to be available in short-lived seasonal patches (Jackson and Caldwell 1993; Ryel et al. 1996). If branching root morphology is an important component of competitive ability for *Elymus multisetus*, it may explain the correlation of CPI to root forks. We would also expect to see a correlation with root tips, but we did not observed one.

In order for adaptive traits to evolve, they must be both beneficial and heritable. Of the three traits correlated with competitive ability, sibling resemblance was detected for 10 day root forks, but not for 10 day R:S or 10 day small diameter root length. In the previously mentioned white clover study, root forks exhibited positive genotypic

correlation and high repeatability (Jahufer et al 2008). Even if the role of root forks in improving competitive ability remains elusive, our detection of both genetic variation in and performance correlation for root forks would suggest that increased root fork production may be adaptive. Lack of family level significance in 10 day R:S and small diameter (0.101-0.200mm) root length may be attributed to a lack of power resulting from low mean sample sizes within families ($n=2$), rather than from a lack of biological significance. In small diameter (0.101-0.200mm) roots, there were marked but non-significant differences between families at 10 days (Figure 5). By comparison, at 50 days, one additional harvested plant per family allowed for detection of family level differences in three root classes and a total of 12 growth traits (Table 1; Figure 5).

Of the CPI-correlated traits, none varied significantly between uninvaded and invaded areas, indicating that these traits have not shifted in response to cheatgrass or that we lacked the power to detect a shift. However, the supremacy of early root traits in CPI correlation would suggest that early resource capture is important in competitive performance of *Elymus multisetus* in cheatgrass-dominated landscapes. More thorough examination of the genetic variation of traits related to early resource capture (e.g. nutrient use efficiency, pulse-initiated root development, root topology) may produce a more mechanistic understanding of adaptive competitive ability.

In addition to our conservative approach for determining adaptation, we conducted a more general survey of growth traits that varied between invaded and uninvaded cohorts, looking for other possible links to competitive ability. Although these traits were not directly correlated with CPI in the presumed gene pool, they may still inform our understanding of the superior competitive performance of invaded

conspecifics (Figure 1; Figure 2). There were significant differences in several inherited growth traits across invasion status (Table 1). Both alone and in competition with cheatgrass, invaded plants were smaller than their uninvaded cohorts. They possessed fewer leaves (Table 1; Table 2), had lower total, root, and shoot biomass (Table 1; Figure 4), and added biomass more slowly. Nonetheless, invaded seedlings were better tolerators and suppressors of cheatgrass competition. The coupling of high competitive ability and reduced size goes against the prevailing theory that greater biomass confers greater competitive ability (Gaudet and Keddy 1988; Keddy et al. 2002). However, our finding that native plants with lower biomass possess greater competitive ability or fitness under cheatgrass competition follows a trend emerging from four separate experiments in the Leger lab (Leger 2008; Goergen, Leger, and Espeland in review; Kulpa and Leger in prep; Rowe and Leger in prep).

There may be adaptive value in being a small plant (Aarssen et al. 2006). In a study of six annual species, greater biomass did not confer advantage in offspring production under competition; in fact, the most fecund species under competition exhibited relatively small biomass in isolation (Neytcheva and Aarssen 2007). Furthermore, for 21 herbaceous annual species, plants from the three, four, or five smallest deciles of plant size accounted for the vast majority of offspring production under competition (Chambers and Aarssen 2009). Furthermore, in drought conditions, there are disadvantages to greater biomass (e.g. high transpiration, tissue maintenance costs) that can reduce survivorship or competitive ability. However, there are other plant-plant interactions besides direct competition (e.g. allelopathy or mycorrhizal interactions) that can result in the appearance of competitive advantage (Fitter 1977;

Allen and Allen 1986; Mahall and Callaway 1992). For example, it is possible that some plants are more tolerant of competition because they release an allelopathic compound or stimulate the growth of soil organisms that inhibit plant growth, rather than excel at resource capture. Although we did not investigate these mechanisms, they may account for our observed disassociation of biomass and competitive ability.

Invaded plants produced smaller seeds than uninvaded cohorts. Within a species, bigger seeds typically produce bigger seedlings, though the effect slackens after development of the first photosynthetic organs (Stanton 1984; Wulff 1986; Kitajima 2007). Our 10 day root, shoot, and total biomass measurements are consistent with this pattern, with larger seeds producing larger seedlings for both invaded and uninvaded sources. Non-genetic maternal provisioning can influence seed size (Eagles and Hardacres 1979; Roach and Wulff 1986). Because of the potential importance of seed size in initial seedling development, maternal effects could be important non-genetic contributors to population differences in seedling survival. Although the mean difference in seed weight between invaded and uninvaded cohorts was less than 10% with substantial overlap in variance, it may have contributed to the larger size and growth rate of uninvaded plants at 10 days. It is unlikely that larger seed size of uninvaded cohorts was due purely to maternal effects; differences in seed size across invasion status persisted in next generation seed collected from plants grown in a common garden, signaling some degree of genetic control. Furthermore, initial seed weight was not correlated with cheatgrass tolerance, cheatgrass suppression, or any 50 or 100 day growth traits. Even if seed weight was maternally-influenced, its importance diminished over time.

We observed small differences in root allocation across invasion status. At all harvest dates, invaded plants produced a higher percentage of fine roots, with significant differences at 50 days (Table 1; Table 2). After 10 days, invaded plants allocated a relatively greater proportion of biomass to root production (Figure 4). There are many examples where root systems with the highest densities are the strongest competitors (Aerts et al. 1991; Casper and Jackson 1997). When competition is mostly for soil resources (e.g. low soil fertility, high water stress), root allocation is a good indicator of competitive ability (Eissenstat and Caldwell 1988; McGraw and Chapin 1989; Tilman and Wedin 1991). Several mechanisms have been suggested to explain the importance of fine roots in competitive ability, including improved opportunistic nutrient foraging (Fitter 1994), adventitious root production in ephemeral nutrient patches (Drew et al. 1973; Bilbrough and Caldwell 1997; Robinson et al. 1999), and enhanced nitrogen uptake at terminal roots (Pregitzer 2002). Increased root allocation in invaded plants may mark the nascent adaptation of root traits, a view bolstered by our previously discussed finding that early root growth traits are correlated with CPI (Figure 6).

While the ability to tolerate or suppress cheatgrass would likely improve lifetime fitness in cheatgrass invaded environments, the need remains to establish a definitive link between seedling competitive ability and lifetime fitness. Because it requires measurement of survivorship and fecundity across several years, evaluation of lifetime fitness for perennial grasses is difficult and could not be quantified in our short-term greenhouse-based experiment. Applicability of conclusions reached through greenhouse experiments to natural systems may be further limited by a genotype-by-environment interaction between the field and laboratory that can distort estimates of natural

heritability (Riska et al 1989). On the other hand, we wanted to quantify below-ground growth traits that may impact overall competitive ability. Certain important performance measurements (e.g. root morphology) are exceedingly difficult to attain in field experiments. If we had undertaken a field experiment, the low survival percentage of seedlings demonstrated in arid systems (Wood et al. 1982; Belknap and Sharpe 1995; Keya 1997; Bowers and Turner 2002) probably would have further restricted our power to detect differences across maternal families and invasion status. For our purposes, first season competitive ability served as a proxy for lifetime fitness. We are currently undertaking field-based evaluation of seedling performance and multi-year fitness for the genotypes used in this experiment to try to solidify the relevance of our findings in natural growth conditions.

For both invaded and uninvaded sources, we observed family-level variation at a high frequency, indicating that many growth traits are likely inherited to some degree (Table 1). Genetic variation of fitness-related traits is the basis for natural selection. Greater genetic variation represents greater evolutionary potential and an increased likelihood for long-term population persistence (Frankel 1974; Stockwell et al. 2003). Populations with low genetic diversity may not possess sufficient evolutionary potential to combat new sources of disturbance, such as climate change, land use changes or new biological invasions (Rice and Emery 2003; Harris et al. 2006). Though not related to measured competitive ability, the level of genetic variation in growth traits that we detected may improve the chances for long-term persistence of *Elymus multisetus* populations in a changing environment.

The ability of native populations to evolve in response to cheatgrass invasion has significant implications for the management and restoration of cheatgrass-invaded communities. Restoration projects often introduce large volumes of seed into an ecosystem which may affect both the quantity and quality of local genetic variation. Commercially available restoration seed material is often derived from a single source population and sometimes even from a single genotype (Young et al. 2003; Shaw et al. 2005). Non-local genotypes can have a deleterious effect on the fitness of local populations (Montalvo and Ellstrand 2000; Waser and Price 2000). There is widespread concern about genetic swamping during large-scale restoration (Montalvo et al. 1997; Hufford and Mazer 2003; McKay et al. 2005). On the whole, the impact of large-scale seeding on the genetic integrity of existing native populations and the ecosystem as a whole remains poorly understood (Knapp and Dyer 1998; Broadhurst et al. 2008). If cheatgrass-invaded populations are rapidly evolving novel genotypes, then the introduction of high quantities of non-local seed near long-invaded populations may reduce both their fitness and evolutionary potential.

Certain current cheatgrass control methods may also pose a risk to evolutionary potential in native populations. Chemical control of cheatgrass involves the use of herbicides that are equally toxic to native bunchgrasses (Whitson et al. 1997; Shaw and Monson 2000). Manual control methods (e.g. tilling, disking, burning) can also damage desirable native species (Mosley et al. 1999). Because of the magnitude of invasion in the Great Basin, the choice for many land managers is not how to eradicate cheatgrass, but where to focus their control efforts to maximize benefit for desired land uses. Managers should consider preserving long-invaded native populations and concentrating

control efforts on more recent invasions where selection pressure on competitive ability may not yet be in operation. Although invaded native populations may lack the habitat or scenic value of more intact grasslands, they may possess important adaptive traits.

Harnessing these adaptive traits is another consideration for managers and restorationists. If long-invaded populations possess greater competitive ability (Figure 1; Figure 2), then seed from invaded populations could be an effective tool in restoring nearby cheatgrass degraded areas. Long-invaded sites should be considered in collection of restoration seed material. However, seed selection need not be limited to long-invaded populations, as there exists significant genetic variation in how seedlings respond to cheatgrass within uninvaded populations (Figure 3). Therefore, screening for inherited traits related to competitive ability and subsequent selection of genotypes exhibiting high tolerance or suppression of cheatgrass in any seed material under consideration may improve its quality. Selection of restoration seed material is often based on seed production in agricultural settings (Young et al. 2003). While fecundity is a large component of fitness, the ability to emerge and persist among cheatgrass may be of greater importance to restoration success in invaded landscapes.

It is still commonplace to select a single seed source or genotype for a restoration project. In the absence of definitive proof for the scale of local adaptation or rapid evolutionary change, we would advocate for the use of restoration seed material with the greatest genetic diversity, rather than gambling on a single genotype. By maximizing seed collection sites and individuals, there is a greater likelihood of capturing a breadth of genetic variation (Guinon 1993; Kitze 1993; Meyer and Monson 1993) and finding some individuals with improved competitive ability. The use of genetically diverse

restoration seed can provide a broad basis for adaptation to future selection pressures, cheatgrass and beyond.

CONCLUSIONS

Evidence is emerging that biological invasions can produce rapid evolutionary change in native plant populations (Callaway et al 2005; Lau 2007; Meador and Hild 2007; Leger 2008). Our findings were consistent with the hypothesis that cheatgrass is exerting a strong selection pressure on native grass populations in the Great Basin. This study provided examples of two methods for assessing potentially adaptive growth traits. The ability to detect genetic variation of traits under selection will help to advance our understanding of the mechanisms of rapid evolutionary change. It can also serve a practical function by informing our management and restoration techniques of native populations in invaded areas.

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TABLES

Traits of big squirreltail plants grown in competition with cheatgrass that are significantly different between invasion status or family. At 100 days, root, shoot, and total weights were only measured for one plant per family; therefore, family was not included as an analysis factor for these traits. This is indicated by “-”. Significant factors ($\alpha=0.05$) are highlighted in bold.

Response Variable	n	Status			Site			Family			
		r ²	F	df	P	F	df	P	F	df	P
Seed weight (g)	991	0.44	69.77	1	< 0.0001	7.72	6	< 0.0001	14.14	42	< 0.0001
Emergence Date	950	0.09	0.08	1	0.7799	1.98	6	0.0664	1.86	42	0.0009
Early growth rate (leaf #)	402	0.47	33.93	1	< 0.0001	23.17	6	< 0.0001	3.04	42	< 0.0001
Early growth rate (leaf length)	402	0.29	11.91	1	0.0006	10.58	6	< 0.0001	1.69	42	0.0065
50 day leaf #	148	0.66	8.42	1	0.0046	12.45	6	< 0.0001	2.48	42	0.0001
50 day root weight (mg)	148	0.54	0.07	1	0.0691	7.30	6	< 0.0001	1.63	42	0.0253
50 day shoot weight (mg)	148	0.66	10.64	1	0.0015	12.31	6	< 0.0001	2.40	42	0.0002
50 day total weight (mg)	147	0.61	7.09	1	0.0091	10.35	6	< 0.0001	1.97	42	0.0033
50 day root total root length (cm)	145	0.48	0.01	1	0.9031	2.44	6	0.0311	1.73	42	0.0146
50 day root diameter (mm)	145	0.60	46.70	1	< 0.0001	3.84	6	0.0018	1.31	42	0.1393
50 day root tips	145	0.49	2.31	1	0.1315	2.72	6	0.0176	1.76	42	0.0123
50 day root forks	145	0.49	0.24	1	0.6238	1.57	6	0.1654	1.90	42	0.0051
50 day (0.000-0.100mm)	145	0.49	0.01	1	0.9700	1.51	6	0.1823	1.98	42	0.0033
50 day (0.101-0.200mm)	145	0.44	1.65	1	0.2019	1.88	6	0.0919	1.49	42	0.0467
50 day (L>0.400)	145	0.64	27.46	1	< 0.0001	8.15	6	< 0.0001	2.08	42	0.0017
50 day % fine roots	145	0.65	40.05	1	< 0.0001	6.46	6	< 0.0001	1.83	42	0.0079
50 day R:S	147	0.50	6.40	1	0.0130	5.74	6	< 0.0001	1.39	42	0.0953
100 day leaf #	482	0.53	61.60	1	< 0.0001	31.58	6	< 0.0001	5.53	42	< 0.0001
100 day root weight	49	0.36	5.00	1	0.0309	3.09	6	0.0138	--	--	--
100 day shoot weight (mg)	50	0.36	6.16	1	0.0172	3.16	6	0.0120	--	--	--
100 day total weight (mg)	49	0.38	5.78	1	0.0208	3.31	6	0.0094	--	--	--

Table 2. Values for growth traits of *Elymus multisetus* plants grown in competition with cheatgrass by invasion status. Mean±SE.

	10 day		50 day		100 day	
	Uninvaded	Invaded	Uninvaded	Invaded	Uninvaded	Invaded
Total weight (mg)	3.10 ± 0.11	3.02 ± 0.11	125.03 ± 11.32	93.94 ± 9.30	215.98 ± 27.56	142.38 ± 25.57
Shoot weight (mg)	2.17 ± 0.08	2.20 ± 0.07	55.67 ± 6.13	34.82 ± 4.83	64.18 ± 7.81	41.89 ± 8.11
Root weight (mg)	0.93 ± 0.06	0.82 ± 0.05	69.36 ± 5.66	58.55 ± 4.94	151.80 ± 20.47	101.23 ± 17.50
R:S	0.49 ± 0.07	0.38 ± 0.02	1.91 ± 0.13	2.25 ± 0.12	2.51 ± 0.15	2.86 ± 0.24
Leaf number	1.22 ± 0.06	1.20 ± 0.06	8.04 ± 0.60	6.32 ± 0.46	10.84 ± 0.36	7.81 ± 0.27
Root Length (cm)	17.12 ± 0.97	16.80 ± 0.87	671.18 ± 44.97	684.00 ± 43.54	1067.57 ± 107.53	1160.37 ± 154.00
Root Tips	49.92 ± 5.12	48.80 ± 3.88	1535.03 ± 130.21	1389.32 ± 120.84	4026.08 ± 467.44	3717.69 ± 538.23
Root Forks	49.22 ± 3.78	51.88 ± 4.87	3361.81 ± 364.69	3926.24 ± 365.90	7143.76 ± 1022.56	8096.50 ± 1537.62
Root Diameter (mm)	0.32 ± 0.01	0.31 ± 0.01	0.26 ± 0.01	0.21 ± 0.00	0.23 ± 0.01	0.21 ± 0.01
Fine root percentage (<0.200mm)	33.58% ± 1.67%	38.77% ± 1.55%	72.05% ± 0.72%	77.96% ± 0.63%	74.30% ± 1.37%	78.08% ± 1.06%
Root Diameter Size Classes (root length in cm)						
0.000-0.100mm	1.86 ± 0.22	1.68 ± 0.15	216.22 ± 20.91	237.86 ± 21.85	423.41 ± 49.67	451.23 ± 66.27
0.101-0.200mm	4.09 ± 0.37	4.92 ± 0.37	269.78 ± 15.24	295.20 ± 14.42	359.96 ± 28.81	451.00 ± 52.80
0.201-0.300mm	2.60 ± 0.19	2.49 ± 0.20	46.24 ± 3.19	49.78 ± 3.15	81.22 ± 8.42	78.60 ± 10.14
0.301-0.400mm	6.37 ± 0.43	5.65 ± 0.37	46.56 ± 3.62	49.34 ± 2.94	80.49 ± 8.79	80.12 ± 9.86
>0.400mm	2.15 ± 0.21	2.03 ± 0.24	90.67 ± 6.86	50.29 ± 5.62	119.38 ± 19.02	96.32 ± 19.74

FIGURE LEGENDS

Figure 1. Relative competitive performance index (CPI) by cheatgrass invasion status. Mean \pm SE. Letters represent significant differences between groups ($\alpha=0.05$). Plants with lower CPI values are better tolerators of competition, showing a smaller reduction in biomass when growth with cheatgrass.

Figure 2. Cheatgrass shoot biomass production when grown with *Elymus multisetus* by invasion status. Mean \pm SE. Letters represent significant differences between groups ($\alpha=0.05$). Lower cheatgrass weight signifies a greater suppressive effect and is seen in plants collected from invaded areas.

Figure 3. *Elymus multisetus* families vary significantly in their effect on cheatgrass weight. Family mean \pm SE. Families are labeled and color-coded by invasion status. Invaded families are labeled ‘I’ and colored white. Uninvaded families are labeled ‘U’ and colored grey.

Figure 4. *Elymus multisetus* biomass traits by invasion status. Mean \pm SE at the three harvest events—10, 50, and 100 days. Asterisks represent significant differences ($\alpha=0.05$) between invasion status. Plants from invaded sites generally exhibit lower biomass and higher R:S.

Figure 5. Family level differences in root length classes at 10 days (left) and 50 days (right) by invasion status--invaded (top) and uninvaded (bottom). Each line represents a different family. Asterisks represent significant differences ($\alpha=0.05$) between families.

Figure 6. 10 day root traits that are significantly correlated with CPI in uninvaded families. Traits are graphed by performance rank. Lower numeric value of rank signifies better performance (i.e. lower CPI, higher R:S, higher root length, or more root forks). Correlation and significance values are reported in text.

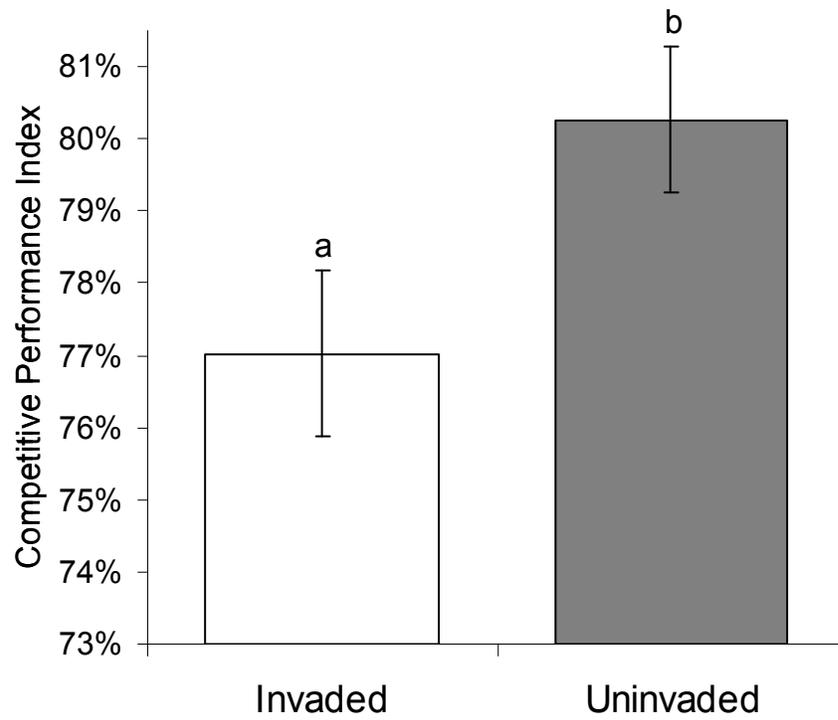
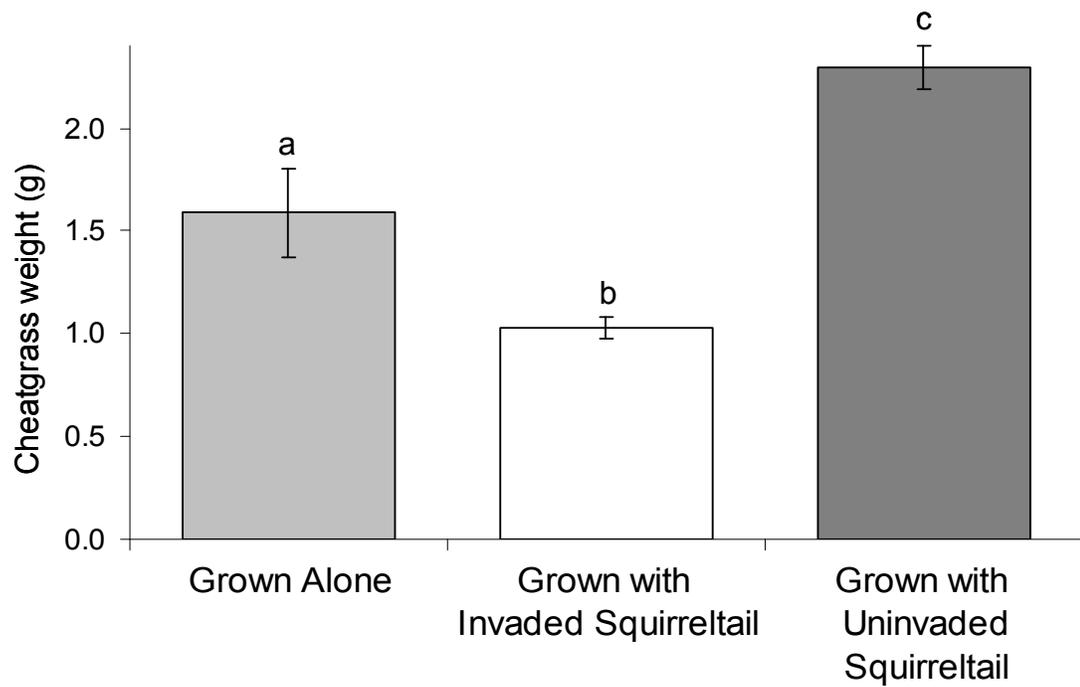
FIGURES**Figure 1.****Figure 2.**

Figure 5

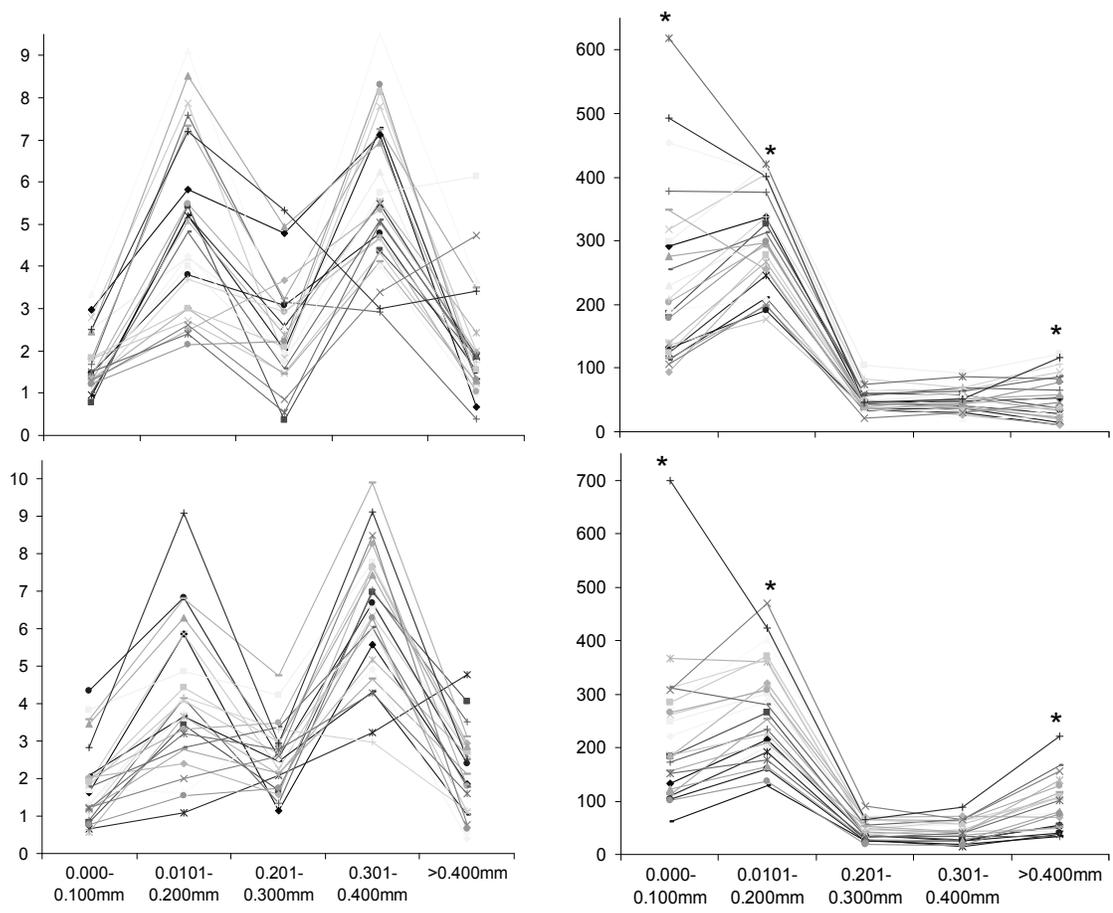
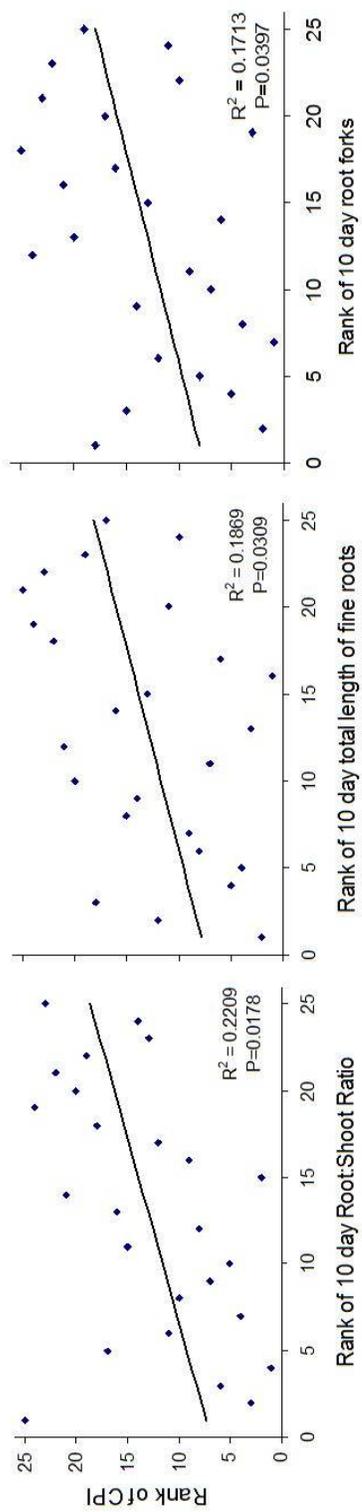


Figure 6



SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Ashley et al. (2003) advocated that land managers consider adopting a policy of ‘evolutionarily enlightened management,’ in which management actions are evaluated in the context of their impact on the evolutionary as well as ecological processes of native populations. Our experiments represent an important step along the path to evolutionary enlightened management by evaluating the potential for native populations to adapt to local or changing conditions in the Great Basin. We found that seed source affected several important seedling performance measures in both field and greenhouse common garden experiments. In the field, locally collected seed outperformed regionally produced commercial seed in seedling establishment through greater emergence and survivorship, indicating that there is likely some level of adaptive genetic variation among populations. In greenhouse trials, seedlings from cheatgrass invaded areas exhibited greater tolerance to cheatgrass competition and a greater ability to suppress cheatgrass than seedlings from adjacent uninvaded areas, which was consistent with rapid evolution in response to selection pressure from cheatgrass invasion.

Evolutionary enlightened management can be applied regardless of the intensity level of desired management activities—from research and passive management to intensive restoration and agronomic development of seed material. Based upon our findings, we think there are several ways in which evolutionary enlightened management could be applied in the Great Basin:

- More reciprocal transplant experiments should be conducted for ecologically important native species in the Great Basin. Reciprocal transplanting could illuminate our somewhat murky understanding of the scale of local adaptation in the Great Basin.

Gradients of elevation, precipitation, and disturbance could inform the development of seed transfer zones, as well as supplement our understanding of the relative importance of selection pressures at work in native populations.

- The connection between squirreltail's competitive ability with cheatgrass and its lifetime fitness should be field tested. While the ability to tolerate or suppress cheatgrass would likely improve lifetime fitness in cheatgrass invaded environments, a definitive link needs to be established before seedling competitive ability can be considered an adaptive trait.
- Additional precision seeding trials with more species at more locations could provide valuable information about seed source related performance. These trials are relatively easy to conduct and can inform management decisions regarding what seed material is most appropriate. Long-term monitoring of such trials could help to quantify lifetime fitness differences between seed populations in a variety of environments.
- Duration of cheatgrass invasion should be considered in the management of native populations. If native populations can evolve in response to cheatgrass, then long-invaded populations may possess important adaptive traits. Managers should consider preserving long-invaded populations and concentrating cheatgrass control efforts elsewhere.
- The use of non-local seed material during restoration should be accompanied by consideration of its effects on local genetic variation. Non-local seed, especially from a single genotype or population, may impact both the fitness and evolutionary potential of local populations. Managers should consider the quality and quantity of genetic variation of available seed material and err towards greater genetic diversity. This may translate to

the collection of local seed from a greater quantity of plants or it may mean purchasing commercially available seed material from multiple source populations to increase the likelihood of capturing a breadth of genetic variation. It may also include the decision not to revegetate certain areas (e.g. long-invaded or genetically unique populations).

– Selection of restoration seed material should include evaluation of field-based competitive ability and other fitness-related traits. Seed for restoration is often selected on the basis of successful seed production in an agricultural setting, and selection pressures that affect performance in a production field may differ substantially from selection pressures in wild populations. Furthermore, while fecundity is a large component of fitness, the ability to tolerate cheatgrass invasion may be of greater importance to the long-term population persistence in cheatgrass-invaded areas.

We are not advocating that a purely non-interventionist approach is necessarily the most appropriate method for preserving evolutionary potential of native populations, only that each management decision should be weighed in the context of its evolutionary impacts. We have provided two examples of methods for assessing potentially adaptive growth traits. The ability to detect genetic variation in traits under selection will help to advance our understanding of the mechanisms of local adaptation and rapid evolutionary change. It can also serve a practical function by informing our management and restoration techniques of native populations in the Great Basin.

Ashley, M. V., M. F. Wilson, O. R. W. Pergams, D. J. O'Dowd, S. M. Gende, and J. S. Brown. 2003. Evolutionarily enlightened management. *Biological Conservation* 111:115-123.