INTRASPECIFIC VARIATION IN *ELYMUS ELYMOIDES* ROOT TRAITS AND ITS INFLUENCE ON COMPETITIVE OUTCOMES

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Abstract

Understanding how root traits vary within and among species, and how they respond to heterogeneous environments, can provide important insight into functional plant attributes that influence plant survival in competitive environments. Selecting plant material with root traits that will support its survival in competitive environments may help improve the outcomes of ecological restoration, yet root traits are rarely incorporated into restoration sourcing decisions. Specifically, the impacts of population-level variation in root traits of restoration material are often overlooked. For these reasons, seven root traits of seedlings from twelve populations of *Elymus elymoides* spp. *elymoides* were quantified using agar as a growth medium. *Elymus elymoides* is a native perennial bunchgrass frequently used in restoration in the Colorado Plateau that has been shown to exhibit significant among-population variation in many above- and below-ground traits. The root growth of all *E. elymoides* populations was compared to growth in seedlings of *Bromus tectorum,* an annual invasive grass found on the Colorado Plateau with a dense fibrous root system that proficiently captures soil water and outcompetes most native plants. Results showed significant variation among *E. elymoides* populations in seedling root length (ANOVA, $p<0.001$) and lateral root number (GLM, $p<0.001$). Two *E. elymoides* populations were chosen for use in a greenhouse study imposing competition from cheatgrass and a water stress gradient. One population (Ashley National Forest, UT, USA collection), whose seedlings had a similar number of lateral roots as cheatgrass, represents direct spatial overlap of soil resources with cheatgrass, and thus has access to similar resources. The second population (Fishlake National Forest, UT, USA collection), whose seedlings had fewer lateral roots than cheatgrass, represents indirect soil resource overlap with cheatgrass. While all seedlings were competitively excluded under cheatgrass competition, a time of death analysis showed that the Ashley population persisted longer ($p=0.029$, $R^2 = 0.028$) than the Fishlake population suggesting that seedling traits such as a more robust root system positively influences seedling survival. The water stress gradient revealed differing allocation strategies and plastic responses to water stress in each population: the Ashley population had higher root length ratio (RLR) and root mass fraction (RMF) and had a stronger plastic response to water stress while the Fishlake population allocated more biomass to aboveground growth (including seed production). These results suggest that populations that allocate more growth to belowground traits (as in the Ashley population) will perform better in restorations where competition from species like cheatgrass is present. However, this presents a practical challenge when trying to produce seeds for restoration because plants that invest more in root growth tend to have lower seed production, as only the Fishlake population produced seeds during the study period.

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Introduction

Root competition and soil resource relations

Roots play an important role by anchoring plants in their substrate, storing photoassimilate, and sequestering resources from their substrate (Schenk 2006). The ability of plant roots to sequester resources has two important limiting factors: heterogeneous availability of resources and presence of neighbors. Resources are variable in an environment due to limited or variable precipitation, soil type and variable or nutrient turn-over (Grime et al. 1994). When resources are variable, the plant must have mechanisms and adaptations in order to overcome limitations and acquire those resources. Many plants have plastic responses to limited resources, where the plant increases allocation to a particular structure such as the increase to root partitioning under nitrogen stress and competition which aids in acquiring more resources (Berendse and Moller 2009).

When focal individuals and neighbors occur sympatrically, and the resource acquisition of the neighbor decreases the available resources of the focal individual, resource depletion competition occurs (scramble competition) (Schenk 1999). This reduction of resources is the competitive effect of the neighboring individual (Cahill et al. 2005). If resource sequestration by neighbors is high, resources available to the focal individual may be reduced below a necessary threshold and they must have a competitive response to attempt to compensate for the reduced resources (Berendse and Moller 2009). Competitive responses can manifest themselves as plastic responses in carbon partitioning to the roots to acquire more resources (Berendse and Moller 2009). Sometimes this partitioning leads to trade-offs of carbon allocation from other structures. For example, *Elymus elymoides* in competition with a high density of *Taeniatherum caput-medusae* led to a decrease in leaf growth rate for *E. elymoides* but a greater root mass (Hironaka and Sindelar 1975). In this study, the growth of the individual is said to be suppressed (out-competed) as it was not able to grow as if the neighbor was absent (Goldberg and Fleetwood 1987). Additionally, the resources of a focal individual can be reduced below the threshold necessary for functioning, leading to mortality (competitive displacement) (DeBach 1966).

Examples of the importance of root competition on plant growth and fitness come from a wide variety of study species and systems. In Mediterranean Spanish forbs, higher root partitioning in dry habitats was directly correlated to survival (Lloret et al. 1999), while in an agricultural field, Gersani et al. (2001) showed that root competition in soybeans causes suppression of less-competitive individuals. In this study, individuals grown alone partitioned more carbon to aboveground growth and reproductive structures while the plants grown with a neighbor shifted carbon allocation to more biomass in root growth (Gersani et al. 2001). Furthermore, Lankinen (2008) provided evidence that *Viola tricolor* had reduced pollen competitivity (slow pollen tube germination) as a result of root competition - which has direct negative fitness impacts.

Heterogeneous environments coupled with competition negatively impact resources availability (Goldberg et al. 1999), and seedlings are particularly vulnerable to these negative impacts, as the establishment period leads to high mortality (Moles and Westoby 2004, James et al. 2011), and establishment is limited by heterogeneous resources (Reichenberger and Pyke 1990, Reader et al. 1993). Further limitations on establishment occur when seedling-seedling competition occurs and growth suppression or mortality results (Moles and Westoby 2004). Elevated vulnerability may also occur when the neighboring individual is an adult and imposes resource depletion competition onto the seedling (Reichenberger and Pyke 1990, Foster 1999). This is important to consider with co-occurring species that have different life-histories.

Traits that determine plant performance under resource heterogeneity and competition can be measured in several ways. Biomass is often used as a key measure of plant performance, but when considered alone, important functional traits can be missed (Svejcar 1990). Additionally, more functionally-specific parameters can be calculated using mass and length of above- and belowground structures. This includes root mass fraction and shoot mass fraction (RMF and SMF; Table 1), calculated by dividing the root mass or shoot mass by total mass, respectively (Funk 2008). These parameters indicate how mass is allocated to structures by the plant (Reich 2002) and can reveal plastic responses to changes in resources, indicating higher resource capture capabilities (Wang and Taub 2010, Reich 2002). Specific root length (SRL), calculated by dividing the root length by the root mass (Craine et al. 2003, Acciaresi and Guiamet 2010), may be indicative of root foraging capacity in the plant; roots with high SRL can access resources deeper in the soil, potentially accessing the water table (Ryser 2006). SRL is also affected by origin environment of the plant, where water/nutrient-limited environments are correlated to a longer SRL (Hajek et al. 2013). Root length ratio (RLR), calculated by multiplying RMF and SRL (Acciaresi and Guiamet 2010) indicates how mass is distributed about the length of the root; a high value is implicated in fast resource capture (Ryser 1995). Generally, a high RMF, SRL and RLR are implicated in drought tolerance (Leguizamón et al. 2011).

At a finer scale, aspects of root architecture and morphology can be measured to assess more specific resource capture abilities (Sorgonà et al. 2005). Root architecture characterizes the spatial proliferation of root axes, while root morphology characterizes the surface features of the root such as lateral root pattern, root diameter (Lynch 1995), and lateral root number (Jacobs et al. 2004). Main root axes (taproots, seminal roots) have absorptive functioning but also are important for placement of lateral roots in soil to forage for resources (Fitter 1996). Lateral roots (first order roots) are associated with water and resource acquisition; longer lateral roots and more numerous roots are associated with higher nutrient acquisition (Sorgonà et al. 2005, Alvarez-Flores et al. 2014). Finally, root tips (root magnitude; additive of main axes and lateral roots (Fitter 1987)) have been documented as a location for phosphorus and nitrogen acquisition, so higher number of lateral roots and main axes leads to root tips and higher resource acquisition as well (Sorgonà et al. 2005). Individuals with similar architecture and function may have access to similar amounts of resources. Root morphological differences among species exist and are evident in differences in root architecture and morphology. Gross et al. (1992) found interspecific variation in age at root branching and number of root tips (main roots and lateral roots), with annual grasses having the highest root tip numbers earlier in ontogeny than any other growth form assayed.

Functional and morphological trait variation

The differences in root functional traits and morphology are known to vary widely between species, and these differences translate to different resource acquisition capabilities (Gross et al. 1992, Craine et al. 2003). For example, the SRL measured in grassland species showed that tall grasses had long root systems with high fine root biomass (roots <1mm) while legumes had smaller root systems that were less able to reduce soil moisture compared to grasses (Craine et al. 2003). Differences in root traits even exist between species in the same genus or growing in the same habitat. For example, Bell and Sultan (1999) found that two species of *Polygynum* had significantly different investments in roots, root deployment responses to favorable sites, and root foraging capabilities. Finally, a study of three grass species growing in rangeland habitat in the western United States found significantly different root lengths for each species, indicating differences in soil exploration ability for resources (Sheley and Larson 1993).

While interspecific differences in root traits have been the subject of decades of research, understanding of intraspecific differences in root traits has received much less consideration (but is increasing in consideration; see Albert et al. 2011). Intraspecific trait variability can be caused by two processes: phenotypic plasticity (described in detail above) or local adaption (Albert et al. 2011). Some of the best research on genetic variability and local adaptation in root traits, and their influence on plant survival, comes from agriculture and silviculture. For example, when drought tolerant and intolerant rice ecotypes were crossed, the first generation had many traits of the drought tolerant parent, including greater root length and thicker roots (Ekanayake et al. 1985). The second generation was similar but had more variability; the drought tolerant parent was hypothesized to have dominant traits that were passed on (Ekanayake et al. 1985). In *Quercus* congeneric species, the number of lateral roots is an important trait to measure because in tree species it is positively and significantly correlated to plant survival (Thompson and Schultz 1995, Kormanik et al. 1997a) and is a heritable trait (Kormanik et al. 1997b).

Intraspecific trait variability can arise from local adaptation to biotic (Ferrero-Serrano et al. 2011), climatic (Oleksyn et al. 2002) and edaphic (Leguizamón et al. 2011) differences that may lead to differences in population characteristics. Some of the best examples of local adaptation come from studies of populations of the same species growing in mesic and dry conditions. For example, the root foraging capacity assessed in two ecotypes of *Chenopodium quinoa* from wet and dry habitats showed rooting behavior of the dry ecotype (longer lateral roots) that was more conducive to absorption of water resources than the short lateral roots of the wet ecotype (Alvarez-Flores et al. 2014). Populations of *Populus davidiana* from wet, dry and mesic environments in China had differing drought tolerances, with the population from dry conditions having the highest water use efficiency and root to shoot ratio (high root mass) (Zhang et al. 2003). Intraspecific variation in root morphology was documented in genotypes of *Triticum aestivum* L., where individuals from dry Mediterranean locales had a greater number of main root axes, indicating higher resource capture abilities and drought tolerance (Manshadi et al. 2007).

In general, species that occupy a wide range of heterogeneous environments (including soil type and precipitation) are likely to exhibit intraspecific trait variability in roots. For example, transplanted *Elymus elymoides* plants from twelve western US locations to an Arizona common garden experiment showed that populations had differing adaptations (Clary 1975). Individuals from water limited environments had low transpiration rates, were short, and had low total biomass compared to those from water abundant environments. The western U.S. provides a particularly interesting heterogeneous landscape to explore how environmental variability impacts intraspecific variation of populations found within it.

The Colorado Plateau and the Invasive Cheatgrass

The Colorado Plateau encompasses parts of Utah, New Mexico, Colorado and Arizona and has an arid climate, receiving between 22 and 64 centimeters of precipitation annually (Abruzzi 1995). These dry conditions support a large array of grasses found in the Colorado Plateau that are adapted to drier climates (Abruzzi 1995). Pinyon- Juniper (Durrenberger 1972) and Sagebrush (*Artemesia tridentata*) constitute dominant plant communities in this area and volcanic soils and sands are distributed throughout the region (Durrenberger 1972). The rangelands are used for livestock grazing (Abruzzi 1995) and many national parks are found in the Colorado Plateau Region.

Native habitat in the Colorado Plateau has been altered by a number of different forces, including livestock grazing, mining, oil and gas exploration, as well as the introduction of invasive species. One of the species that is potentially increasing in threat to Colorado Plateau habitats is *Bromus tectorum* L (cheatgrass) (Bradley 2009). Cheatgrass is an annual grass native to Europe that has come to dominate regions of the American West (Stewart and Hull 1949). It grows in dense monocultures that act as biofuel increasing the frequency (from 60-110 year to 3-5 year cycles) and intensity of fires (D'Antonio & Vitousek 1992, Whisenant 1990). Post-fire soil conditions are high in nitrogen and this leads to cheatgrass plants that are larger and more fecund, perpetuating the invasion cycle (Chambers et al. 2007). Cheatgrass invasion in previously uninvaded areas alters trophic interactions; bacteria, fungi and invertebrates are negatively impacted by cheatgrass invasion (Belnap and Philips 2001). It also reduces the colonization of beneficial arbuscular mycorrhizal fungi to roots of some native plants, reducing productivity (Owens et al. 2013).

In addition to altering ecosystems to which it invades, cheatgrass alters resource availability through competition. Cheatgrass germinates earlier than many other native plants and has more rapid root extension at low soil temperatures, giving it early access to more resources (Harris 1967). It also has low diameter roots conducive to a high growth rate which build towards a dense fibrous root system (Harris 1967). Cheatgrass has been documented as suppressing the growth and reducing the fecundity of adult perennial grasses (Goergen et al. 2011, Humphrey and Schupp 2004) and forbs (Parkinson et al. 2013). It does so by greatly reducing the soil water content and the root growth of sympatric native species (Melgoza and Nowak 1991). Additionally, cheatgrass requires 60% more water than *Agropyron desertorum* to produce the same biomass (Hull 1963), highlighting its competitive ability for resource capture even in scarcity.

For these reasons, there is a need for habitat restoration in habitats invaded by cheatgrass. Current restoration approaches including introducing a mix of native (and sometimes non-native) species, often after fires, in the hopes that the seed will germinate and compete with cheatgrass. However, restoration carried out this way in cheatgrass-invaded habitat often fails (James and Svejcar 2010). If not all populations are equal in root traits and competitive ability, as is evidenced by examples above, then it is possible that selecting plant material specifically for root traits that are known to enhance competitive ability may improve restoration outcomes. (Mealor and Hild 2006, Leger 2008, Bossdorf et al. 2009).

Understanding intraspecific trait variation in plant material used in ecological restoration may allow restoration practitioners to select sources that are most likely to be effective competitors in invasive-plant dominated habitats and improve restoration outcomes. For these reasons, I used two approaches to assess inter-population differences in root morphology and functional traits and their implications under water stress and competition. First, I carried out a common garden study with two different nutrient levels to quantify the root morphology (lateral root number and main axes number) of seedlings from twelve populations of *Elymus elymoides* from the Colorado Plateau relative to seedlings of cheatgrass. The number of lateral roots is an important trait to measure because in tree species it is positively and significantly correlated to plant survival (Thompson and Schultz 1995, Kormanik et al. 1997a) and this correlation may be evident in *E. elymoides*. Second, I selected two accessions similar and dissimilar to cheatgrass (based on seedling lateral root number and root length results from the common garden study) to assess survival in competition with cheatgrass and water stress tolerance in juvenile plants seedlings from each *E. elymoides* accession in a greenhouse experiment. With these two approaches I addressed the following hypotheses.

Common Garden Study

Hypothesis 1: Intraspecific variation in root morphology and length will be found.

Hypothesis 2: Nutrient level will impact root morphology and length, but some measures will show greater plasticity than others.

Hypothesis 3: Some populations will have root morphologies and lengths that more closely resemble that of cheatgrass than others.

Competition Experiment

Hypothesis 4: Seedlings with differing root morphology and length (determined in the common garden study) will have differing survival responses to competition with cheatgrass.

Hypothesis 5: Plants from different populations will have differing allocation strategies and plastic responses to water stress which may be related to source environment. Length

Materials and Methods

Study species: Elymus elymoides

Elymus elymoides ssp. *elymoides* (Raf.) Sweezy, (common name, squirreltail, synonym; *Sitanion hystrix* (Nutt.) J. G. Smith)) is a short-lived perennial bunch grass native to the United States (Young and Miller 1985). This fire tolerant species responds positively to prescribed burning by increasing in biomass and shoot density in the following year (Young and Miller 1985). High ecotypic variation has been documented in multiple traits of *E. elymoides* taxa (Clary 1975, Jones et al. 2003, Parsons et al. 2011, Kulpa and Leger 2012). For example, *E. elymoides* plants transplanted from twelve western US locations (AZ, CO, NV, NM, SD, UT, NE) to an Arizona common garden experiment showed that populations from water limited environments had low transpiration rates, were short, and had low total biomass, indicating lower resource demands (Clary 1975).

Adult *E. elymoides* plants also provide the benefit of aiding the establishment of other native plants by suppressing cheatgrass which acts as a soil resource sink in the spring (Booth et al. 2003, Humphrey and Schupp 2004). Second year *E. elymoides* facilitates native establishment by suppressing the growth of cheatgrass, and the increased resources in the reduction of cheatgrass creates favorable conditions for other neighboring plants (Booth et al. 2003). For these reasons, *E. elymoides* is a good candidate for use in an intraspecific root trait variation study with implications for restoration.

Seed Collection

From 2004 – 2011 *Elymus elymoides* seeds from ten populations were wild collected from locations throughout eastern Utah by the US Forest Service for common garden studies as part of the Colorado Plateau Native Plant Program. An additional population was wild collected from Colorado in 2011, and one accession was commercially purchased in 2012 for use in this study (Table 2).

Cheatgrass seeds were wild collected in 2012 near DeBeque, Colorado and in 2013 from Rio Mesa Center, Utah. Storage conditions for the U.S. Forest Service and commercial accessions varied, but the Colorado accession of *E. elymoides* and both cheatgrass collections were stored at 15°C and 16% relative humidity.

Common Garden Study

To evaluate hypothesis one and two, seeds from all 12 *E. elymoides* accessions and one cheatgrass population (from near DeBeque, CO) were germinated on a 1.5 percent agar solution in 13 x 100 mm test tubes (modified from Ellis and Kummerow 1982) with two nutrient treatments: 1) high nutrient (half-strength Murashige-Skoog solution, Sigma Aldrich), and 2) no nutrients. Each accession and nutrient treatment had 20 replicates, each randomized in 13 blocks (test tube racks). Agar was used as a growth medium as it is transparent and therefore allowed non-destructive root data to be recorded. Two seeds were placed on the agar in each tube to increase the chances of having a germinant but only one seed was allowed to remain upon germination. Previous germination trials on commercially available seed from Duchesne, UT showed that *E. elymoides* had a higher germination response with moist cold stratification (unpublished data) so all tubes were cold moist stratified for four weeks at 3°C until germination was observed. These tubes were moved at three or four day intervals to a growth chamber with conditions that simulated the spring conditions of DeBeque Colorado (25.5°C/15°C and 15%/6% relative humidity on 12 hour day/night cycle). Individuals that did not germinate were treated as missing values.

Initial root length was measured when the seeds were transferred to the growth chamber. After 21 days of growth, plants were removed from the agar and final root length (length of the longest root) was measured. These data were used to calculate root growth rate (root elongation rate) as follows:

$$\text{Root Growth Rate} = \frac{\text{Final length} - \text{Initial length}}{\text{Days of growth}}$$

Harvested seedlings were then carefully mounted on sheets of paper, allowed to dry for 24h at 23 ºC and then scanned using an Epson expression 10000XL scanner (Epson). At 400% magnification, the number of main root axes and lateral roots were recorded (Figure 1). The plants were then separated into root and shoot components, dried at 23 ºC for seven days, and then the above- and below-ground biomasses were recorded.

A linear mixed effects analysis of variance (LME ANOVA) with maximum likelihood method was used to assess the differences between means and the impact of nutrient level and accession (with block held as a random effect) on root length and elongation rate. ANOVAs were performed on dry root and shoot mass data (root and shoot mass square root transformed to meet assumptions of normality). These analyses were done to compare intraspecific variation of the accessions (ANOVA was performed with and without cheatgrass data for interspecific comparisons to evaluate prediction three). Then a Tukey-Kramer Honestly Significant Difference (Tukey's HSD) test was performed.

To evaluate hypothesis two, a generalized linear model (GLM- using the quasipoisson method for overdispersed data) was performed on root morphology count data (main root axes, lateral roots, and root tip number) to compare intraspecific variation among *E. elymoides* accessions, and to compare interspecific variation between *E. elymoides* and cheatgrass. All statistical analyses and computations were performed using R statistical software (version 2.15.2).

Competition and Water Stress Experiments

Two *E. elymoides* accessions were chosen for use in a competition experiment and water stress experiment. *Elymus elymoides* populations with seedling root morphology (number of lateral roots) and root length similar and dissimilar to cheatgrass (accessions Ashley and Fishlake respectively) were selected. The competition experiment was performed to evaluate hypothesis four and was carried out in a greenhouse at the Chicago Botanic Garden (Glencoe, IL, USA). Thirty pots with an experimental density of 275 cheatgrass seeds were established for 12 weeks prior to the addition of six *E. elymoides* germinants from each study population (Ashley and Fishlake; see Figure 2). *Elymus elymoides* seedlings were germinated on agar for 8 weeks at 3°C, and then implanted at randomly-assigned locations in the cheatgrass-established pots (competition experiment under water stress) or cheatgrass-free treatments (water-stress experiment). During the first 12 weeks, the cheatgrass was exposed to the photoperiod of 9.5h/14.5h and a day night temperature of 19Cº/17ºC. A 14h/10h (day/night) photoperiod was employed upon the planting of the *E. elymoides* and for the remainder of the experiment. Pots were watered every four days and weekly 20-10-20 fertilizer (NPK 237 ppm) was applied. Additionally, the greenhouse was sprayed with pesticides to control aphids.

To address hypothesis five that the selected *E. elymoides* accessions exhibit plastic root responses, two types of heterogeneous substrates were used in 21cm x 24cm round pots. Sand and gravel were used to replicate the sandy loam consistency found in the Colorado Plateau (Durrenbeger 1972). The presence of root-impenetrable gravel reduces the water available to roots relative to sand (Martre et al. 2002) so thirty pots with 3:1 sand:gravel (by pot volume) and thirty pots with 1:3 sand:gravel (imposing higher water stress) were used. The substrate in each pot was homogenized by hand. Both soil mixtures were used in the competition experiment and water stress experiment. Pots were randomized into blocks and rotated on the bench tops every five weeks to lessen position effects.

The soil water content of each substrate type was measured to note differences in soil water between the substrate treatments. Three 15 mL soil samples were collected from five pots of each substrate type. These samples were weighed while wet, dried at 60°C for seven days then weighed. The mass differences (water content) were recorded and an ANOVA was performed to assess differences in the amount of water held in each substrate type at watering.

Elymus elymoides plants were the target plants and were monitored weekly. The following calculations and data were collected:

Competition Experiment: All *E. elymoides* germinants in the cheatgrass and soil type treatments died by week 6, so a time of death analysis (survival analysis) was performed on weekly survival status (dead or alive) of *E. elymoides* individuals. Water stress and accession identity variables were considered as well.

Water Stress Experiment: To address hypothesis five, at the end of the water stress experiment (14 weeks) *E. elymoides* plants were harvested and roots were gently separated and washed away of sand. The aboveground mass (crown, leaves, culms and inflorescences), belowground mass, final shoot height and root length were recorded. The RMF, SRL, and RLR were calculated. Linear mixed effects models were performed for RMF, SRL and RLR, root length, shoot height, root mass and shoot mass with block and density (varied as a result of mortality) held as random effects. Numbers of leaves were counted and a GLM was performed (using the quasipoisson method for overdispersed data). RLR and SRL data were log transformed and shoot mass data were square root transformed to fit assumptions of normality. All GLM, ANOVAs, time of death, and LME models were selected by backwards elimination for the most appropriate model using R statistical software (version 2.15.2).

Results

Common Garden Study:

Germination

The percent germination response varied by accession (Tables 3, 4). Cheatgrass had the highest germination percentage (100%) followed by Montrose (97.5%) and Fishlake (97.5%) populations. Fishlake II had the lowest percent germination response followed by UPII (32.5%) and UP I (27.5%). Additionally the days to germinate differed by accession ($p<0.0001$).

Cheatgrass germinated the quickest (mean of 8 days), followed by Manti (mean of 13 days) (Table 3; Figure 3).

Plant Dry Mass

The shoot mass of 21 day-old seedlings of the thirteen accessions differed by nutrient treatment (DF= 221, p<0.0001; Table 4) but not by accession or the interaction between nutrient treatment and accession. The shoot mass of seedlings under high nutrients was 0.074 ± 0.01 mg and 0.033 ± 0.002 mg under low nutrient treatment. The root mass of the seedlings did not differ by accession or nutrient treatment (Table 4), with an average seedling root mass of 0.0016 ± 0.001 mg.

Root length and elongation rate

A linear mixed effects ANOVA on the root elongation rate of the seedlings and root elongation differed by accession (DF=218, p<0.0001; Table 4) but not nutrient level or their interaction. The fastest growing accession, Manti had root elongation of 4.6mm/day followed by Fishlake with a rate of 4.46 mm/day (Figure 4). Accession UP I was the slowest, increasing its root length by 2.2mm per day followed by cheatgrass growing at 2.75 mm/day.

A linear mixed effect ANOVA on seedling root length differed by accession (DF=213, p<0.001; Table 3). The results from the Fishlake II population are not presented here due to low germination and high mortality. The accession with the mean longest root (Manti) had root length of 95.93 mm, followed by Fishlake with a length of 93.73 mm (Table 3). Accession Dixie II had the shortest roots of 54.57 mm followed by cheatgrass at growing at 57.54 mm.

Seedling Root Morphology and Length Comparisons

The GLM on the number of main axes did not differ between accessions or nutrient levels, with main axes numbers ranging from one to six (Table 4). The GLM on the number of lateral roots with and without cheatgrass (analysis with cheatgrass reported here) revealed that number of lateral roots differed by accession ($p<0.0001$) and nutrient treatment ($p=0.016$; Table 4, Figure 6). Lateral root proliferation was higher in the low nutrient treatment. In general, Ashley population had the highest number or lateral roots, followed by cheatgrass and Dixie II. The commercial accession had the fewest lateral roots, followed by the Montrose accession.

Greenhouse Experiment

Soil Moisture Content

The soil moisture content of the substrate type showed that the 1:3 sand:gravel soil type held 60% less water ($p<0.0001$) than the 3:1 sand:gravel soil type (1.4 and 3.4g of water respectively).

Competition Experiment

A time of death analysis with a Weibull distribution was performed on the persistence of Ashley and Fishlake seedlings growing with adult cheatgrass and revealed that survival differed by accession ($p=0.029$) (Table 5). Ashley seedlings had a higher survival proportion than Fishlake (Figure 7). Ashley population exhibited a type I survival curve where most mortality occurred late: 50% of planted individuals were still alive three weeks after planting. Fishlake population exhibited a type II survival curve where most mortality occurred early: only 20% of all planted individuals were still alive by week three.

Water stress experiment

Linear mixed effects ANOVA showed that root length did not differ by water stress level or accession (Table 5). However, water stress, but not accession, significantly explained variation in root mass ($p=0.003$) and SRL ($p=0.009$). In general, plants had higher root mass, lower SLR, and more leaves in the high water stress treatment (Table 5, Figures 8, 9, 10).

Accession, but not water stress, significantly explained variation in RMF ($p<0.001$). Ashley plants had higher RMF than Fishlake plants (Table 5 and Figure 11). Finally, accession significantly explained variation in leaf number ($p=0.04$) and shoot mass($p<0.001$), with water stress marginally significant at $p=0.063$). Shoot mass was highest in high water stressed Fishlake accessions, and lowest in Ashley low water stressed accessions (Figure 12). Additionally, accession ($p<0.001$) significantly explained variation in RLR, with water stress marginally significant at $p=0.052$). RLR was highest in low water stress and higher in Ashley than in Fishlake accessions (Figure 13). Finally, twenty-two Fishlake juveniles produced between one and three inflorescences (some of which produced seeds) while only one Ashley juvenile produced a single inflorescence (Figure 14).

Discussion

Significant intraspecific variation in root traits was found among studied *E. elymoides* populations in the common garden study, and these differences may translate to better survival under competitive conditions. In particular, seedlings with a greater investment in roots, (particularly number of lateral roots; see Figure 6), had higher survival under competition with cheatgrass. Ashley seedlings had approximately 3 times more lateral roots than Fishlake plants, under low and higher nutrients, and they survived the longest in competition with cheatgrass (Figure 7). Other studies have found that these root traits can influence seedling survival under water limited conditions. This is supported by previous studies showing that lateral roots are important characters for seedling survival (Thompson and Schultz 1995 and Kormanik et al. 1997a), as they are implicated in substrate resource capture (López-Bucio et al. 2003, Sorgonà et al. 2007) and resource exploration (López-Bucio et al. 2003).

Intraspecific variation in root morphology and length

A number of potentially important root traits were found to vary significantly among populations, even when overall root mass did not. This included root length, root elongation rate, and number of lateral roots (Table 4). This suggests that studies measuring only root mass may be missing important morphological characters that vary among populations. Because these differences were identified in a common environment, it is likely that at least some of the variation measured is due to genetic differences, and may therefore be a result of local adaptation to biotic or abiotic conditions at the source site (Albert et al. 2011). In fact, studies have shown that higher root tip number (additive of main root axes and lateral roots) is directly correlated to higher resource capture (López-Bucio et al. 2003) and higher root length may be related to root resource foraging (Ryser 2006). However, some of these differences may be a result of maternal effects (due to environmental differences between source populations) rather than genetic differences (Bischoff and Mueller 2010).

Plastic responses to different nutrient levels

Seedlings showed no plastic responses to differing nutrient levels root mass, root length (Table 3), or elongation rate, (Figure 4), suggesting that these measures, at least in seedlings, may be under genetic control. Number of lateral roots differed by accession and nutrient level, with more lateral root proliferation under low nutrient treatment than high nutrients. Here, all accessions displayed foraging capacity when nutrients were sparse, with Ashley showing the greatest plastic response in the increase in lateral roots at low nutrients (see Figure 6). These results disagree however, with those of Sorgonà et al. (2005) who reported higher lateral root (root tip) proliferation under high nitrate supply in citrus cultivars. Several strategies could be at play here: the plants response of increased lateral root proliferation under high nutrients may be an attempt to acquire as many resources as possible while available. However, the plants response of decreased lateral root proliferation under high nutrients may be avoiding unnecessary energy expenditure of constructing more root structures while resources are readily available. Conversely, the plants' response of increased lateral root proliferation under low nutrients may be indicative of high foraging capacity as the roots seek sparse resources in the substrate. However, the plants response of decreased lateral root proliferation under low nutrients may be avoiding energy expenditure of constructing more root structures that will need to be sustained in a resource limited environment while resources are not guaranteed to the plant.

Shoot mass also showed a plastic response to nutrient level (Table 4; Figure 5) via increasing shoot mass production with increasing nutrients. The ability to effectively assimilate nutrients is a beneficial trait in heterogeneous environments when nutrients are sparse.

Comparison with cheatgrass

The root traits of some accessions of *Elymus elymoides* more closely resembled cheatgrass than others. Fishlake and Manti had the longest roots while Dixie II and cheatgrass had the shortest roots (Table 3). This suggests higher foraging capacity in Fishlake and Manti, and lower foraging capabilities in Dixie II and cheatgrass. Cheatgrass did have the second highest horizontal root spread measured, with only one *E. elymoides* accession exceeding it (Ashley accession had a mean of 16.1 and 11.8 lateral roots (high and low nutrients respectively); see Figure 6). The Ashley population may therefore have overlapping resource capture with cheatgrass due to similar horizontal root proliferation strategies (sensu Parrish and Bazzaz 1976). All other accessions had less horizontal spread than cheatgrass.

Competition experiment

The two selected accessions with different seedling root morphology and length differed in survival responses to competition with cheatgrass. While no seedlings planted into pots of dense cheatgrass survived beyond 6 weeks, a time of death analysis shows that a higher proportion of plants from Ashley were able to survive longer than those from Fishlake (Figure 7). Results of the common garden experiment provide some insight into which root traits may be providing a survival edge for Ashley plants: Fishlake seedlings had slightly longer roots, but Ashley seedlings had more than 3 times more lateral roots than Fishlake seedlings (and even more lateral roots than cheatgrass; Figure 6).

It is possible that the Ashley seedlings were more effective at accessing resources because of their high number of root tips (Sorgonà et al. 2007). Furthermore, increased lateral root number may influence establishment, and factors other than early root elongation (Harris 1977, sensu Eissenstat and Caldwell 1988), as seen in Fishlake, may be important as well. Number of lateral roots is positively and significantly correlated to plant survival (Thompson and Schultz 1995, Kormanik et al. 1997) however this needs to be evaluated further in more grass and forb species.

Goldberg (1996) argued that recording the competitive response of seedlings to established plants is an important measure of estimating competitive success. While seedlings from both accessions were competitively excluded by cheatgrass, seedlings from Ashley had better competitive success than Fishlake by having a higher proportion of surviving individuals. These results are corroborated with Kormanik et al. (1997b), which showed that *Quercus* congeneric seedlings with few lateral roots were less competitive than individuals with more lateral roots.

It is not clear if the differences in root traits and competitive ability between the Ashley and Fishlake accessions are the result of local adaptation or not. Seed used in this study was wild-collected, so differences seen may be at least in part due to maternal effects (Bischoff and

Mueller 2010). While the germination response varied by accession, this may have been a function of seed age and storage; for example the low germination of the UPI and UPII accessions may be explained by the fact that they were collected 9 years prior to use in this study. However, the difference by accession in amount of time needed to germinate may point at different dormancy and germination requirements (Baskin and Baskin 2004) that are influenced by local adaptation. In addition, very little is known about the competitive environment of the original source populations. However, based on these results it may be predicted that there is more competition (either via cheatgrass or other native and non-native species) at Ashley than Fishlake, and the *E. elymoides* growing at Ashley have adapted to this more competitive environment. Numerous studies have identified similar intraspecific differences that are likely the result of local adaptation when different populations or genotypes are grown in competition with other species. This includes a 2011 study by Leguizamón et al. *Sorghum halpense* from sub humid (drier) Argentinian populations had higher relative growth rate (mass) at drought conditions higher competitivity (higher yield) than populations from sub humid locations. The humid populations had higher performance under higher water conditions. This gives evidence that competitive relationships vary by population and are impacted by source environment.

Water stress experiment

The specific root length (SRL) of both accessions was affected by water stress level, with a higher SRL in low water stress treatments (Figure 10). Higher SRL indicates higher soil exploration (Hajek et al. 2013) and higher resource capture, as it means the roots are thinner and can permeate small pores and access deeper water tables (Leguizamón et al. 2011, sensu Lynch 1995, Ryser 2006). Higher SRL is also associated with drought tolerance (Bell and Sultan 1999, Leguizamón et al. 2011) and could be a beneficial trait when considering restoration material in semi-arid environments. While these results run counter to predictions for plants growing in water stressed environments, lower SRL (as seen in both accessions in the high water stress) (Figure 10), is associated with higher compacted substrate penetrability (Fan and Yang 2011) and longer life span (Adams et al. 2013). These characteristics can both be beneficial in dry environments, as drying soil becomes compacted and energy expenditure to structures is costly in limited water and nutrient resources. Additionally, longer life span of roots increases time of resource capture in those roots. Overall, high SRL as seen in Ashley is indicative of foraging capacity of roots while a lower SRL influenced by high water stress in both accessions is indicative of durable roots (see functional trait table, Table 8).

The two accessions displayed differing root mass fractions (RMF) regardless of water stress, with Ashley having a larger RMF than Fishlake (Figure 12). Larger RMF in Ashley suggests that allocating more mass to roots provides better access to more water and nutrient resources (Wang and Taub 2010, Leguizamón et al. 2011) and has more successful outcomes in belowground competition (Acciaresi and Guiamet 2010). Ashley therefore, has greater access to resources than the Fishlake population. The RLR differed by water stress, and by accession, which was higher in Ashley accession and at the low water stress (Figure 14). This is indicative of short-lived roots with fast resource capture (Ryser 1995). Fishlake population with its lower RLR values may have longer lasting roots (Table 8). Differences in RLR are driven by its components (RMF x SRL). Ashley had a higher RMF and a higher SRL at low water stress and lower SRL at high water stress. These SRL and RMF results are similar to those seen in the common garden experiment (data not shown) which suggest that Ashley has greater soil resource exploration and capture abilities at early and juvenile stages, than Fishlake as the root system had higher relative carbon allocation. High RLR is also related to low root tissue density which has a short life-span (Ryser and Lambers 1996). This also may be related to higher root surface area and drought tolerance which are beneficial in water limited environments (Bell and Sultan 1999). In all, higher SRL, RMF, and RLR are related to drought tolerance (Leguizamón et al. 2011) and these values are seen in Ashley juvenile plants grown in the greenhouse.

While shoot height did not differ by accession, the mass of aboveground structures differed, with Fishlake allocating more mass to its shoots. Additionally, there was a plastic response of higher shoot mass with higher water stress. This response varied by accession with Fishlake having larger shoot mass (Figure 13). Larger aboveground shoot mass observed in the higher water stress treatment may be beneficial in supporting root growth through photoassimilate provisions (Weaver and Himmel 1929). Fishlake juvenile plants also produced more inflorescences and seed production than Ashley (Figure 15). The relationship between aboveground growth and belowground growth is an important one to consider (Grime et al. 1994) as nutrients and water are needed for light resource capture physiology and photoassimilate sugars are needed for root growth [and growth of the whole plant]. However, as evident in Ashley, having smaller leaves is advantageous in resource limited environments as the water and nutrient demand on the root structure to support the shoot structures is lower (Clary 1975, Zhang et al. 2011, Alvarez-Flores et al. 2014). Kulpa and Leger (2012) found that drought caused selection for individuals with smaller aboveground mass of *Elymus elymoides* spp. *californicus* with higher survival. A similar drought history may have occurred in the Ashley source population.

Rowe and Leger (2010) showed increasing root:shoot ratio (higher root allocation) in more competitive *Elymus multisetus* individuals from cheatgrass dominated cites. These results may be useful for collection of *Elymus elymoides* plants in the Colorado Plateau as excavation of these grasses is rarely feasible.

Questions that remain

Intraspecific differences in root traits identified in this study extend only to the seedling and juvenile stages of growth; these differences may increase or disappear as plants grow to adults. The potential for ontogenetic differences in lateral root production and any other trait measured between populations exists and its consideration in experiments is important (Coleman et al. 1994, Reich 2002). While seedlings from the Ashley population had many more lateral roots than any other accession, it is possible that this difference will disappear as seedlings age and other populations develop lateral roots. However, understanding the timing of critical plant development stages is important as it may influences survival. Ashley's 'early' lateral root proliferation appears to provide it with a competitive advantage when grown with cheatgrass.

Numerous studies have shown that not only lateral root number but also lateral root length is important in predicting competitive outcomes, as it is associated with higher resource capture with longer lengths (Sorgonà et al. 2007). In this study, lateral root length was not measured in either the common garden study or competition and water stress study (and lateral root number could not be measured in the competition and water stress study) so it is unknown whether this measure was impacted by nutrient level or accession. Future studies in this system would benefit from explicitly measuring this character, but the challenges of measuring lateral roots when plants are grown in a soil potting medium are significant.

In studies such as this, it is also important to understand whether any plastic responses identified are driven by water stress or resource acquisition by neighbors (or some combination of both) (Callaway et al. 2003). Of the traits that differed by water stress level (root mass, shoot mass, SRL, and RLR), the random factor, density, was not significant in any these traits. These results show that plastic responses can be attributed to water stress caused by the soil texture, and interactions between *Elymus elymoides* conspecifics on an intraspecific level likely did not have an impact on mass allocation.

While results of the common garden experiment appear to translate to the competition and water stress study (for example, differential investment in lateral root proliferation of individuals in an accession influencing survival), this was not always the case: root length was significantly explained by accession in the common garden study, but no accession-level differences were found in the competition and water stress study (Tables 6 and 7). The extent to which these differences were due to different growing media and environments versus the measurement of different life history stages (21 day old seedlings vs. 105 day old juvenile plants) is not known. However, other studies have shown important differences just based on the growing media alone. For example, Hargreaves et al. (2009) reported delayed lateral root production in barley (*Hordeum vulgare* ssp. *vulgare* and ssp. *spontaneum*) with the use of two percent gel observation chambers as compared to plants growing in soil. It is unknown if similar impacts occurred in the *Elymus elymoides* seedlings reported here, but a similar study comparing soil and agar to the same-aged seedlings would provide insight.

Finally, the cheatgrass used in this experiment may itself show intraspecific variation in important root traits that would impact its competitive interactions with introduced *E. elymoides* seedlings. This may limit the application of study results to all sources of cheatgrass. However, the competition and water stress study used cheatgrass that was collected from two populations (one in Colorado and one in Utah), and in general low genetic variation has been documented among cheatgrass populations (Meyer and Leger 2010). Additionally, because such a high density of cheatgrass was employed in this competition experiment, individual variation was likely negligible (Mead 1979) as the resource draw-down by each individual becomes summed.

Implications

Selecting plant material in *E. elymoides* for seed production in restoration of post-fire disturbance currently focuses on selecting source material with high aboveground mass and high seed output. However, these results suggest that seedling survival may be related to root structure, and traits important to resource capture vary between populations. An accession chosen here for study (Fishlake), invested highly in aboveground structures, but yielded seedlings with a higher mortality proportion than another accession (Ashley) which invested more in belowground structures. Cahill and Ericksson (2006) performed a study to typify growth suppression in a competition experiment between adult and seedlings. The authors note that an allocation pattern, such as the one reported in this study, may suggest that investments in above ground mass is a reproductive effort and investments in survival traits is an investment in survival tactics (Cahill and Ericksson 2006).

Some follow up studies could include exploring whether these patterns in varying root structure appear in other species of the Colorado Plateau. Also, to explore whether these patterns in seedling root structure can be observed using conditions closer to nature, could be beneficial as well. Finally, heritability estimates of lateral root orders were high in five *Quercus* species (ranging from 0.66-0.92) (Kormanik et al.1997b) and exploring the adaptive potential of these root traits could be important for restoration practices that seek to create communities that persist. Seedling quality (successful survival) and growth with outplanting (Duryea 1985) relative to variation in root traits has been investigated in tree species for forest restoration (Duryea 1985, Lloret et al. 1999, Jacobs et al. 1999), but is very limited in grasses and forbs that are the target of rangeland restoration Furthermore the variability within a population for measured traits needs to be assessed in populations, as selecting live individuals for certain traits then outplanting is time consuming and costly when restoration is performed at the landscape scale.

Conclusion

Understanding root functioning is a critical aspect of selecting seed to broadcast for restoration in arid and semi-arid environments. There are many elements such as heterogeneous resources and competition with neighbors that limit success. A goal of this study was to investigate an approach to identify early seedling root morphology in *E. elymoides* that may be indicative of survival. There is evidence that this practice provides research practitioners with an outlet to identify suitable restoration material that will persist in communities. The link between seedling survivorship and root structure was explored; with one population (Ashley) having a higher lateral root proliferation and persisting longer when grown with cheatgrass. Additionally, root and shoot traits important to plant functioning vary between accessions, with Ashley investing more carbon to root growth and Fishlake investing more carbon aboveground. Selecting plant material with higher belowground allocation than aboveground allocation has practical limitations on seed production for restoration, though it may improve restoration outcomes by increasing individuals that establish in invasive dominated and arid landscapes.

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Figures

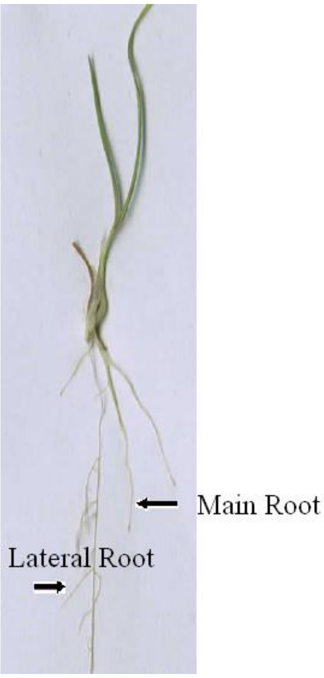

Figure 1. Root morphological parameters measured (cheatgrass seedling) (illustrated on a digitized cheatgrass seedling from the common garden study).

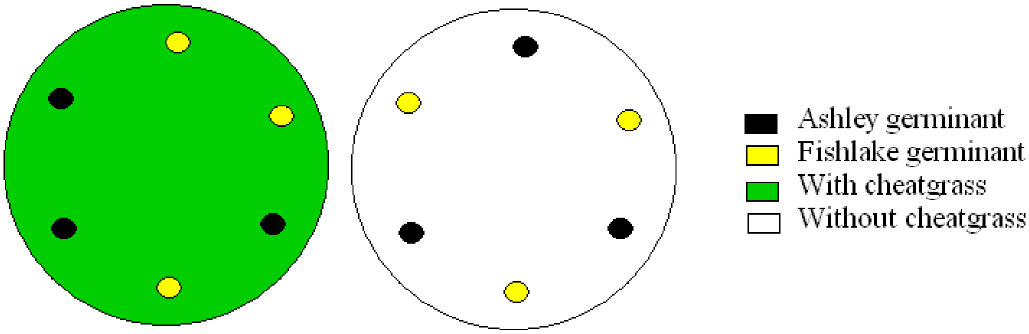

Figure 2. Seedling orientation in 21.4 cm diameter pots in the greenhouse. Seeding method was repeated for both substrate types (1:3 sand : gravel and 3:1 sand : gravel), and *E. elymoides* seedlings were randomly assigned to a location for each pot.

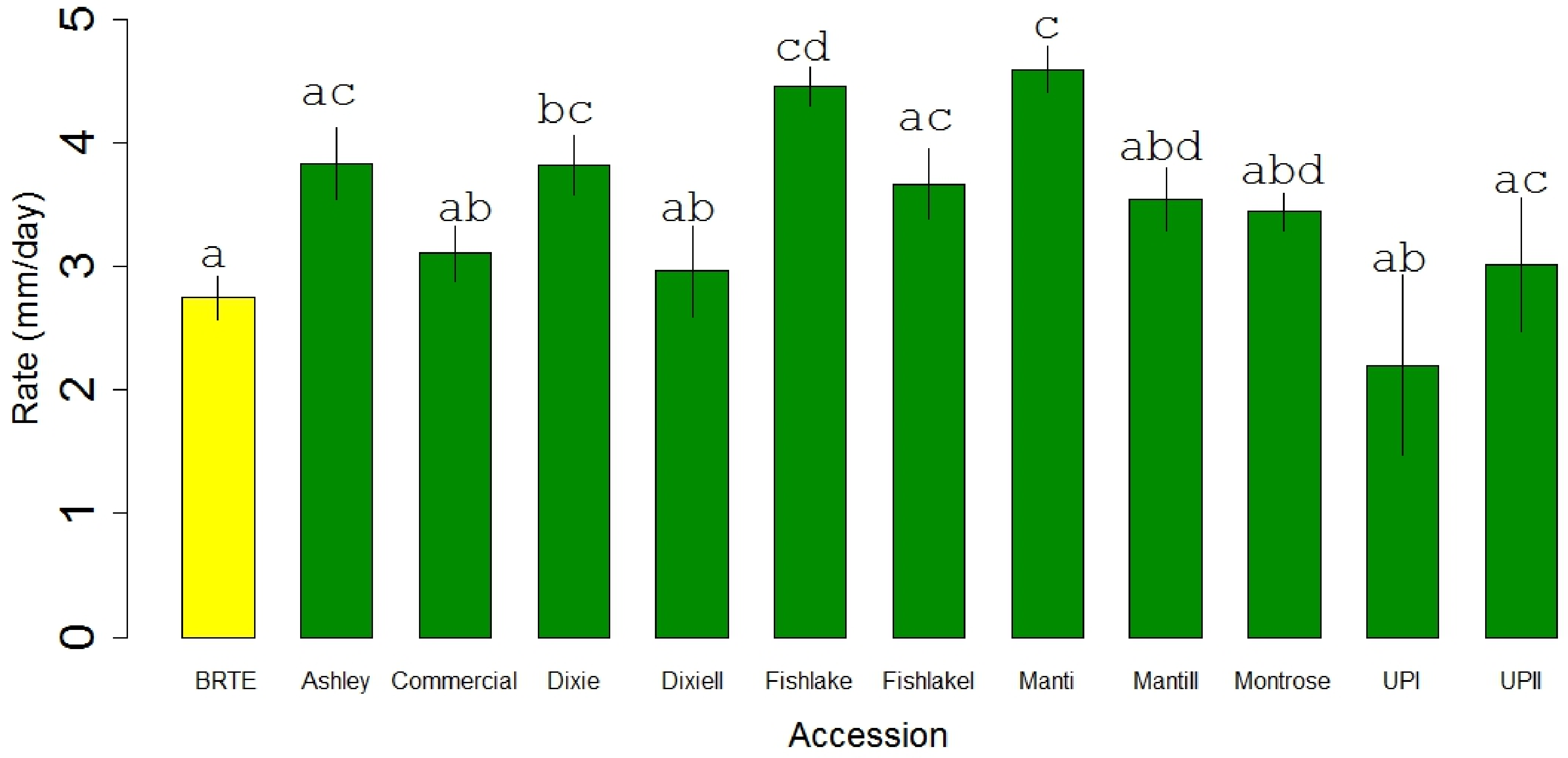

Figure 4. Mean root elongation rate, measured on longest root axis of 21 day-old seedlings (*Bromus tectorum* in yellow). Values are mean ± SE.

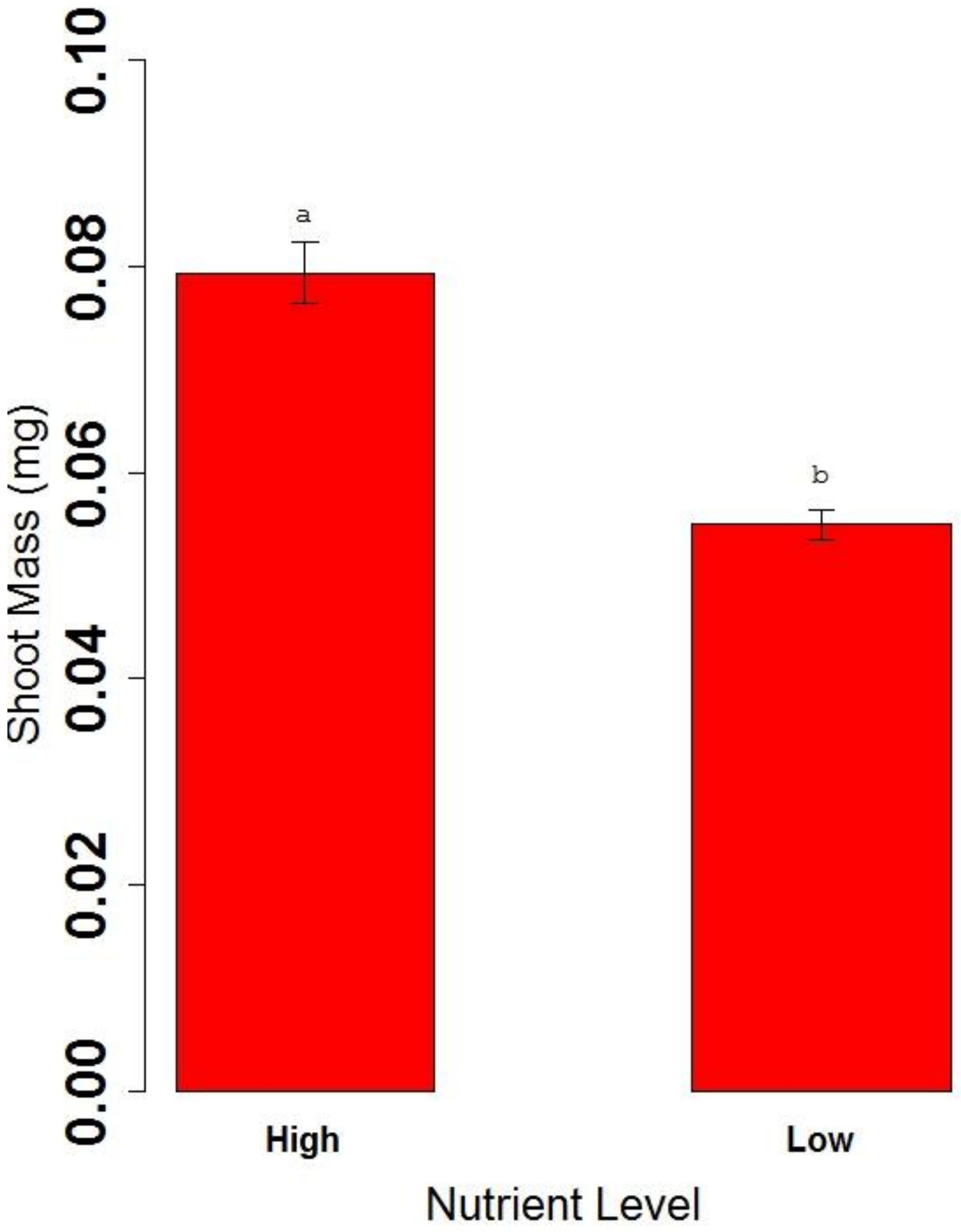

Figure 4. Mean shoot mass (mg), measured on longest root axis of 21 day-old seedlings. Values are mean ± se. Letters denote significant differences between groups.

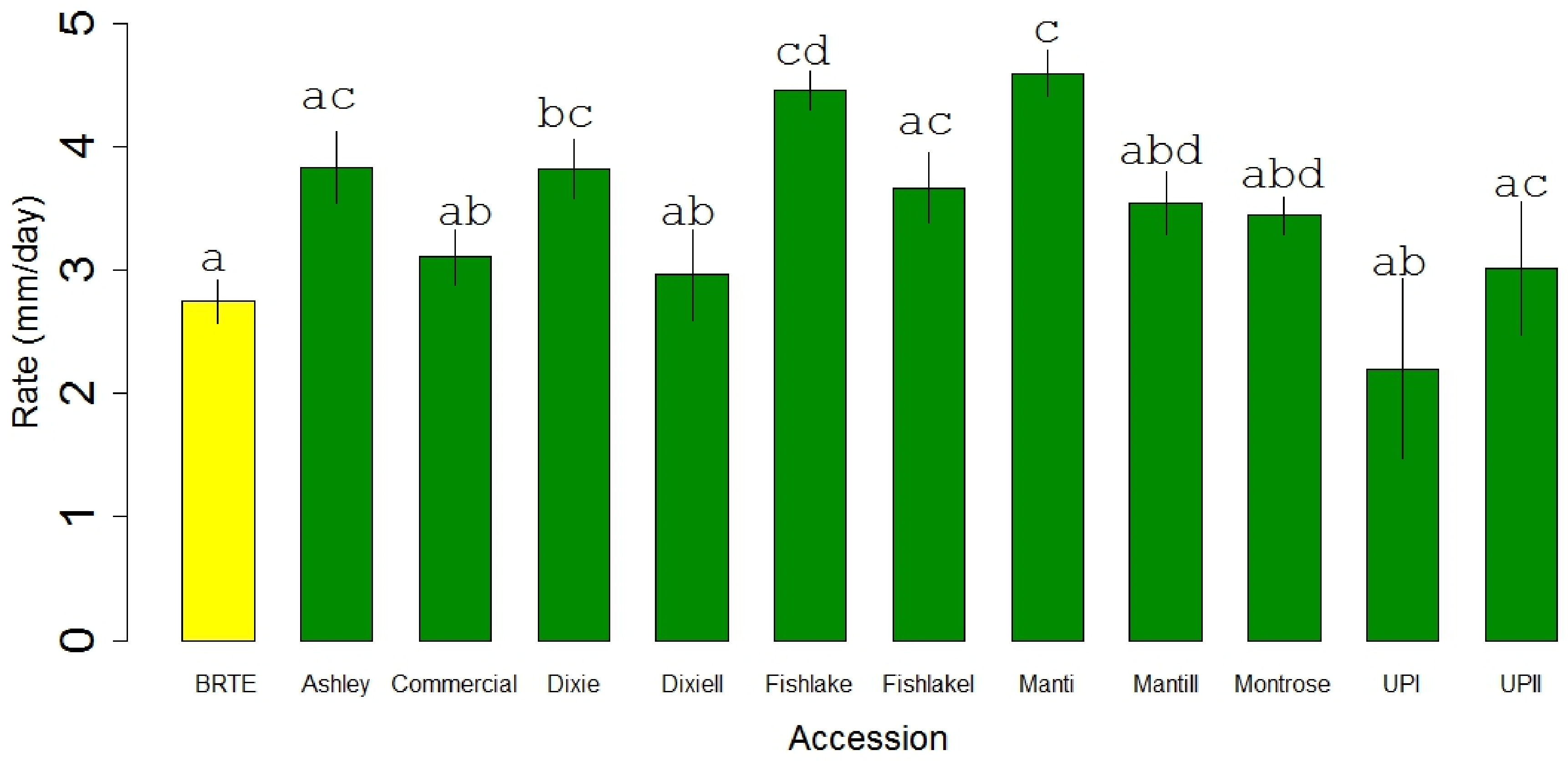

Figure 4. Mean root elongation rate, measured on longest root axis of 21 day-old seedlings (*Bromus tectorum* in yellow). Values are mean ± SE.

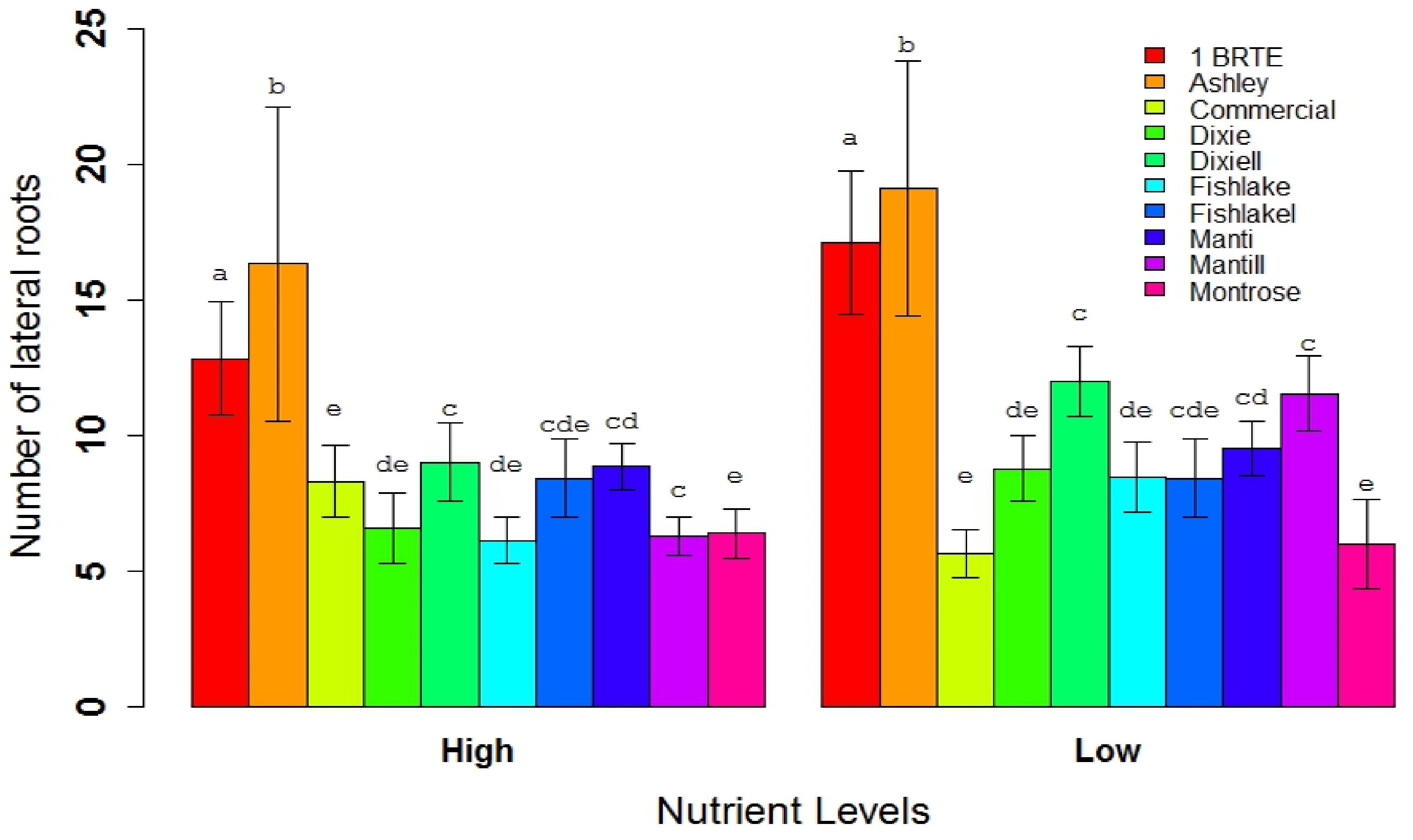

Figure 6. Mean lateral root number of *E. elymoides* populations and *B. tectorum* 21 day-old seedlings. Values are mean ± SE.. Letters denote significant differences between groups.

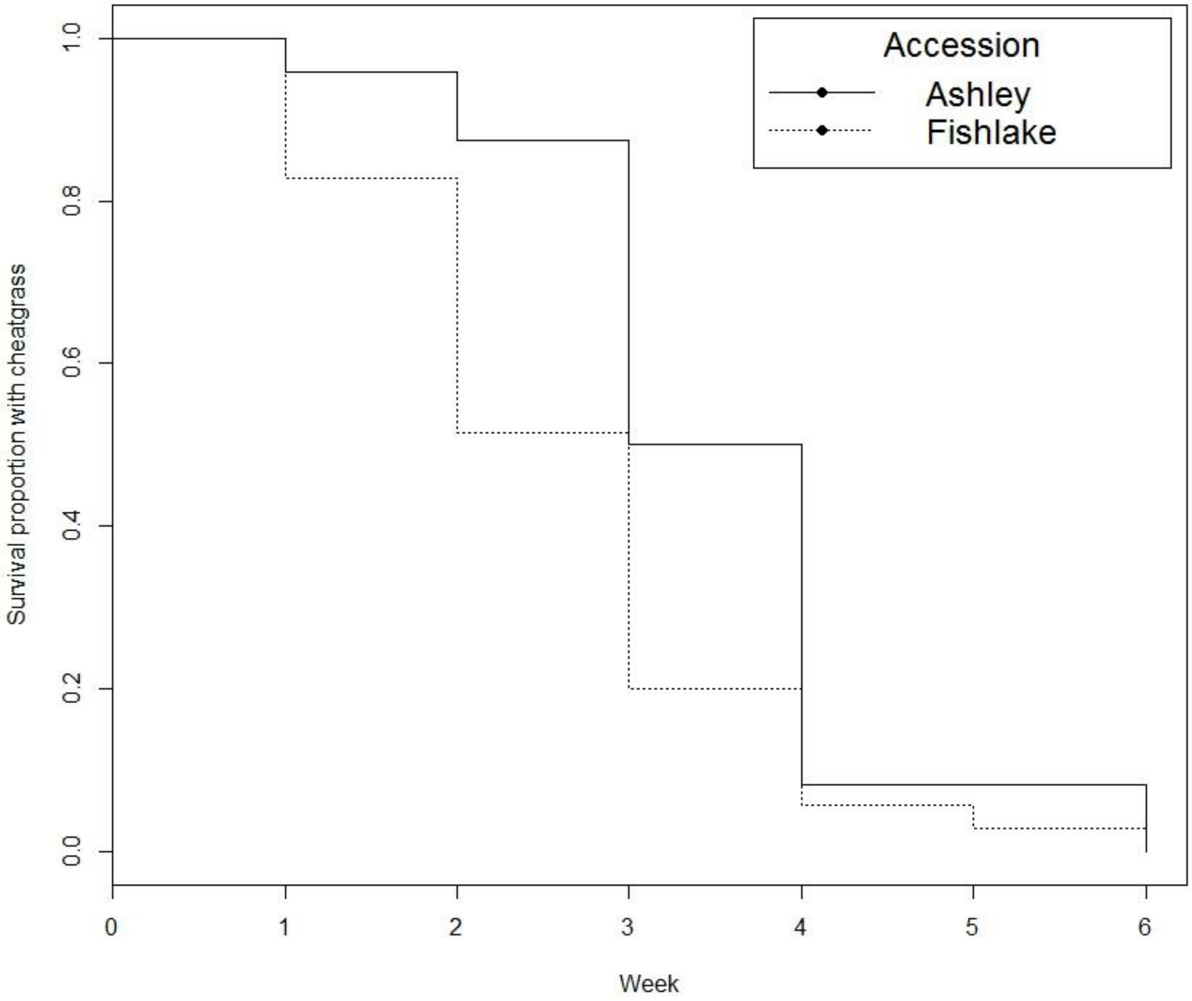

Figure 7. Significantly different survivorship curves (p=0.029) for two populations of *Elymus elymoides* seedlings growing with adult cheatgrass plants in a greenhouse competition study.

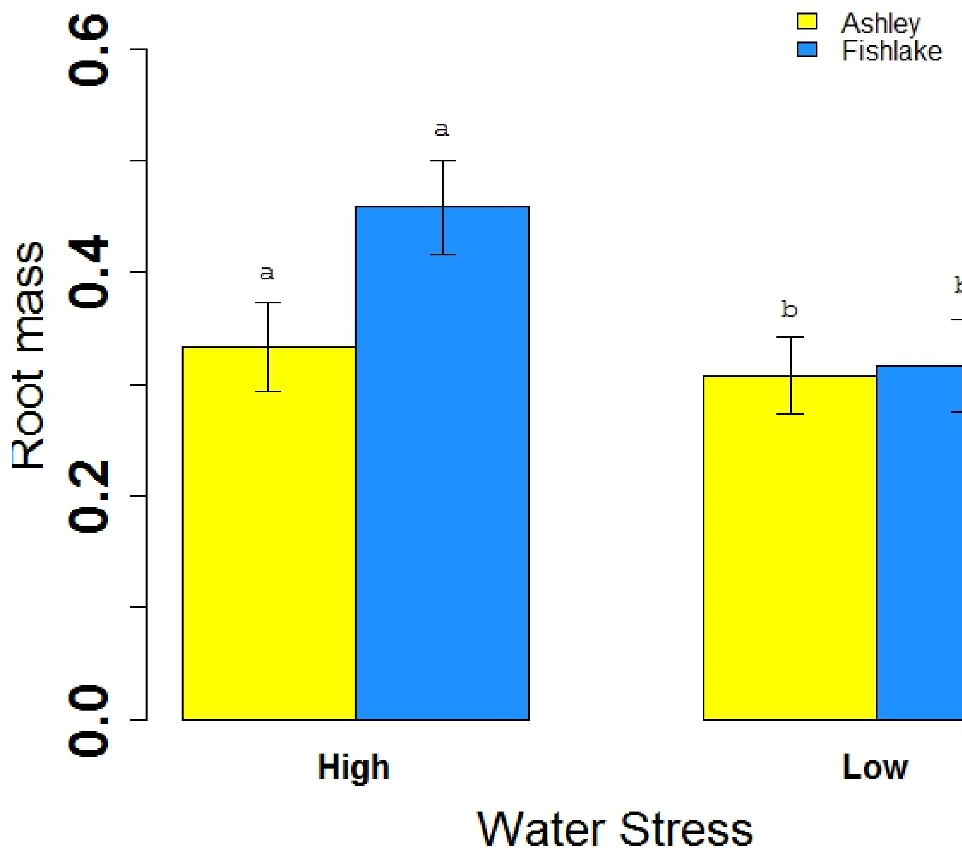

Figure 8. Mean root mass (mg), measured on roots of 105 day-old *Elymus elymoides* juveniles. Values are mean ± SE. Letters denote significant differences between groups.

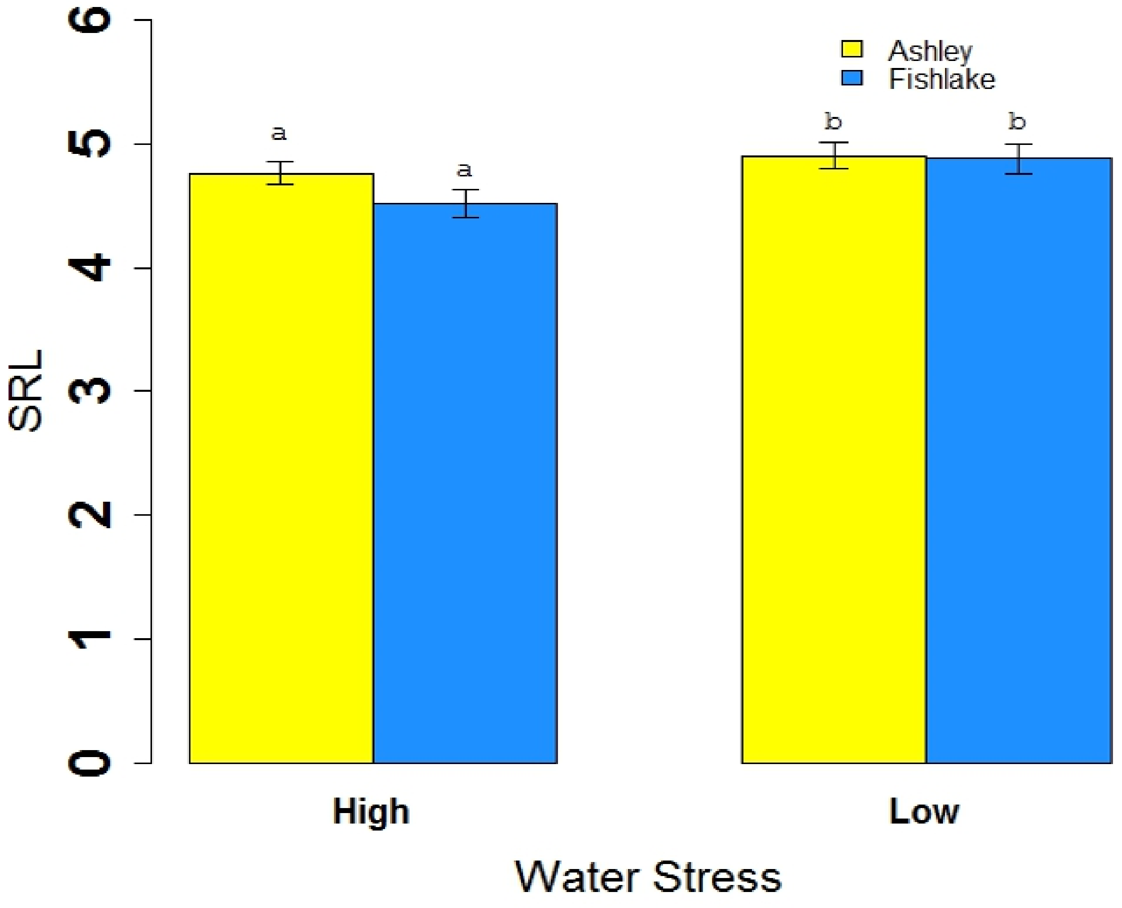

Figure 9. Mean SRL (g root / cm root), measured on roots of 105 day-old *Elymus elymoides* juveniles. Values are mean ± SE. Letters denote significant differences between groups.

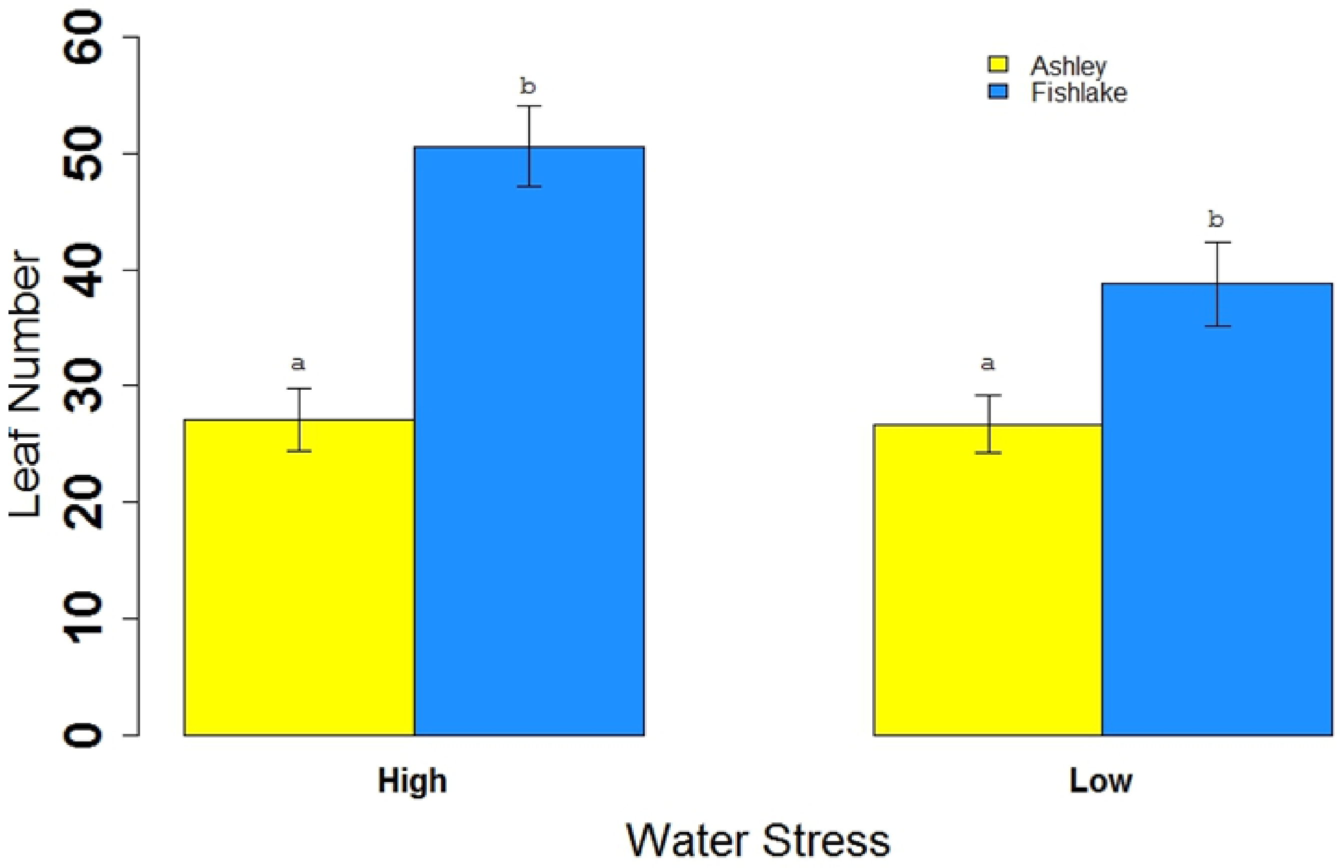

Figure 10. Mean number of leaves, measured on shoots of 105 day-old *Elymus elymoides* juveniles. Values are mean ± SE. Letters denote significant differences between groups.

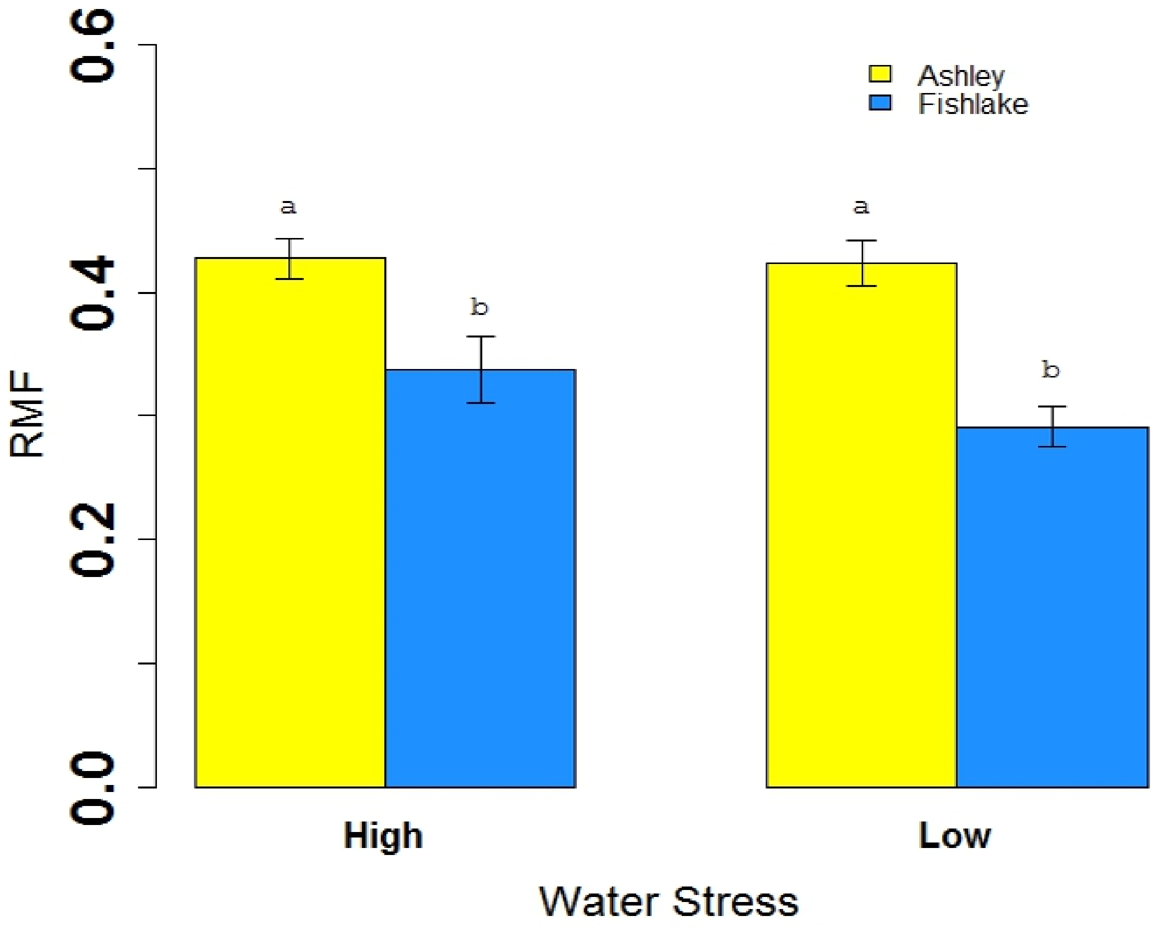

Figure 11. Mean RMF (root mg / total mg), measured on roots of 105 day-old *Elymus elymoides* juveniles. Values are mean ± SE. Letters denote significant differences between groups.

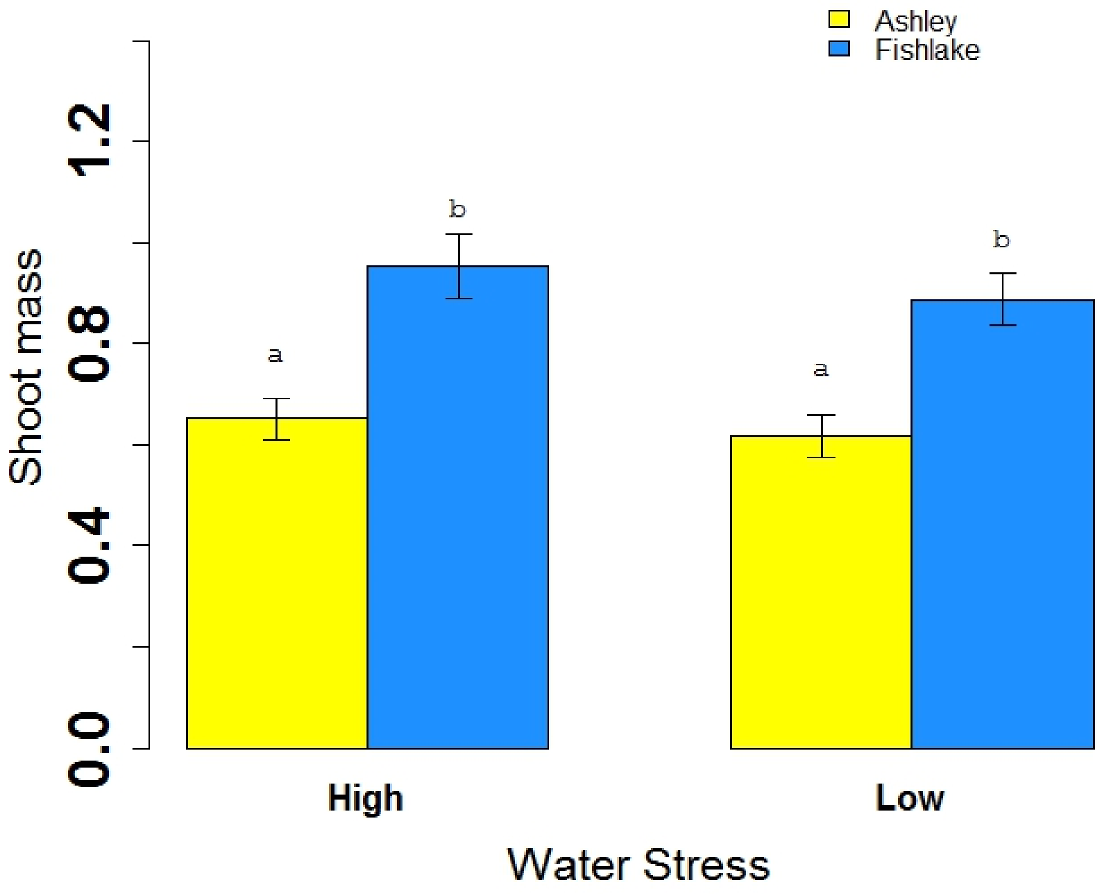

Figure 12. Mean shoot mass (mg), measured on shoots of 105 day-old *Elymus elymoides* juveniles. Values are mean ± SE. Letters denote significant differences between groups.

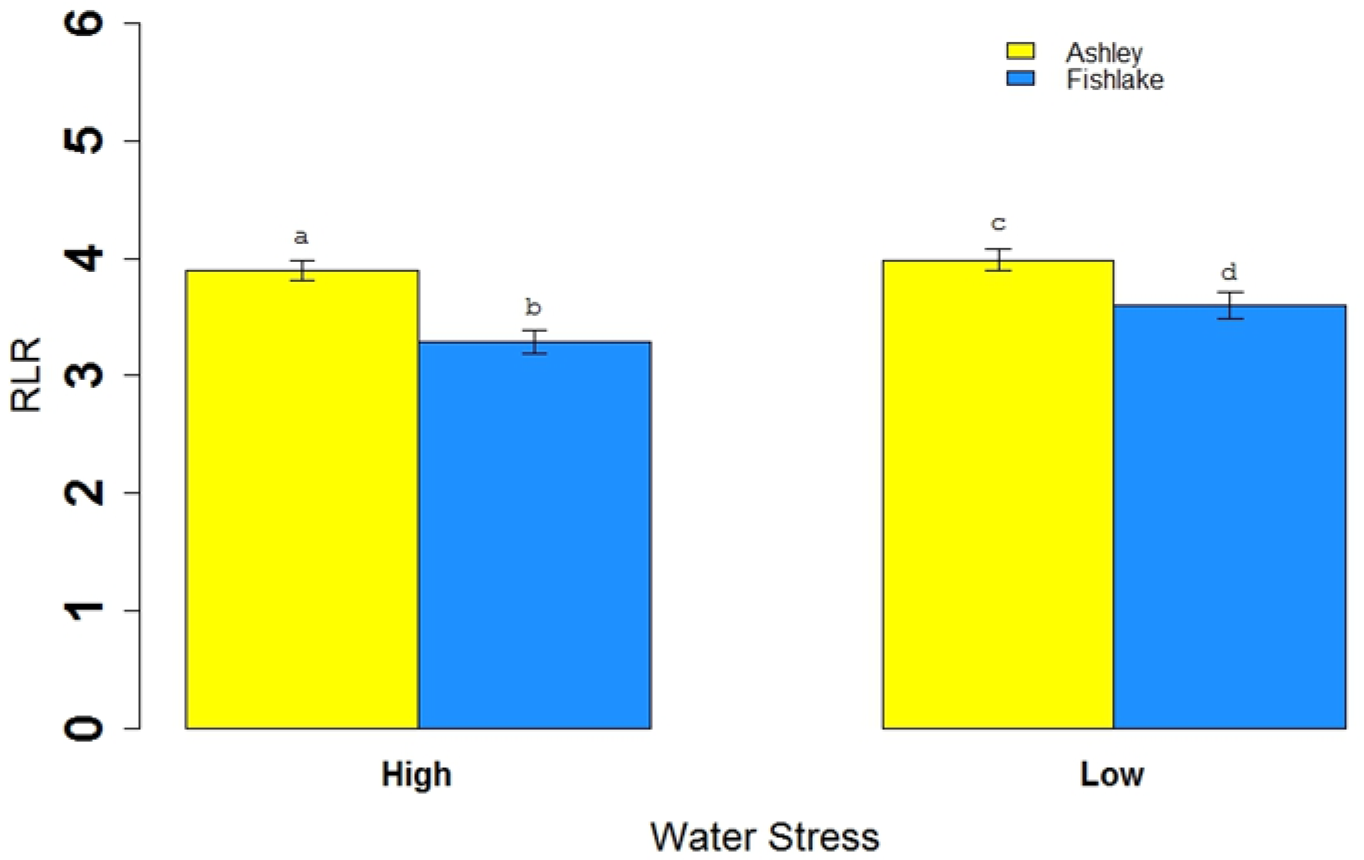

Figure 13. Mean RLR (RMF x SRL), measured on roots of 105 day-old *Elymus elymoides* juveniles. Values are mean ± SE. Letters denote significant differences between groups.

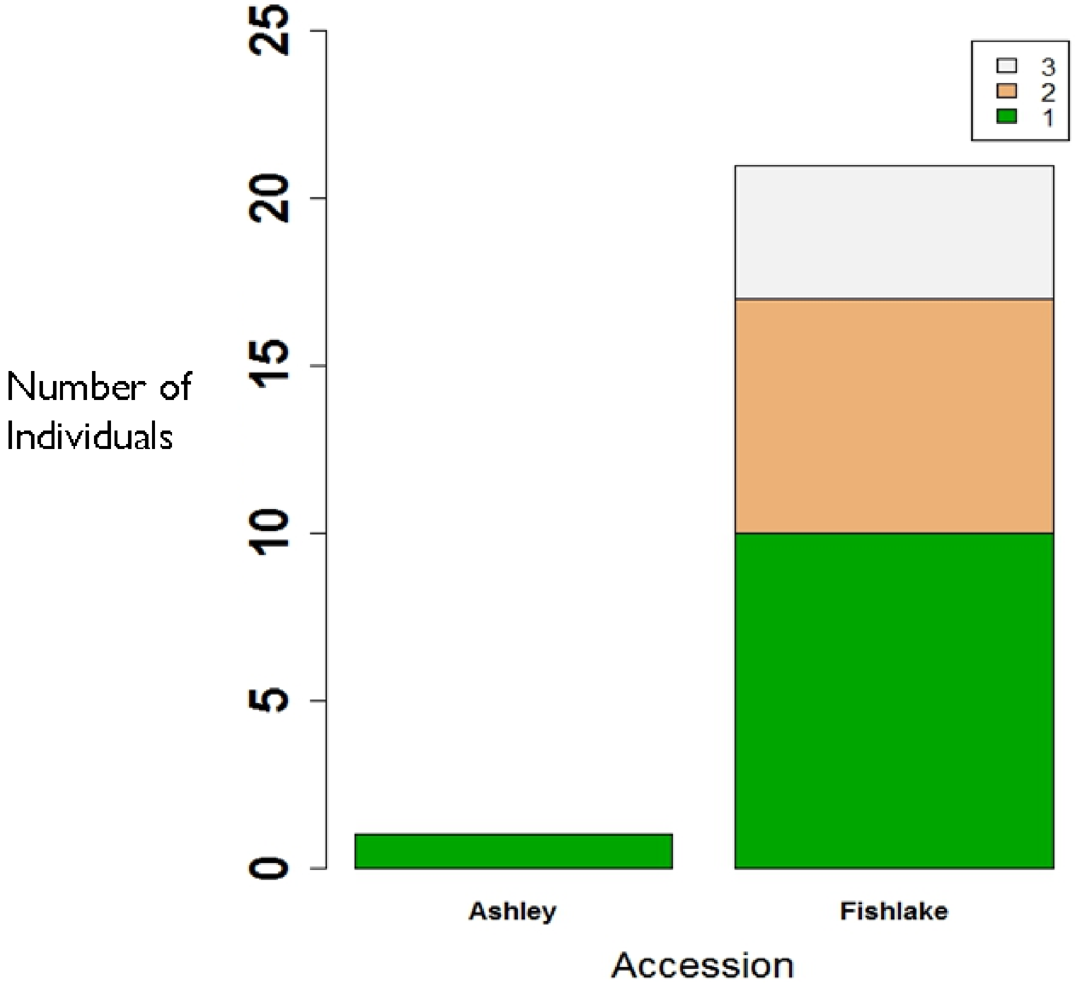

Figure 14. Count of individuals from each accession with inflorescences (colors represent number of inflorescences on each plant, ranging from one (green) to three (grey), counted on shoots of 105 day-old *Elymus elymoides* juveniles. Values are of observed data.

Tables

Table 1. Table of root functional traits, calculations and implications for plant performance.

Trait	Response			
	Higher value	Implications	Lower value	Implications
Root mass fraction (RMF) Root mass/total plant mass	-Response to reduced resources[1] -Larger absorptive area for resources	-Higher access to water and nutrients[2] -Greater belowground competitive ability[3]	Smaller absorptive area for resources	-Less resources acquired
Specific root length (SRL) Root length/root mass	- Higher soil exploration[4] - Higher resource capture[5] - Thinner roots that can permeate small pores - Can access deeper water tables[6,7] - Drought tolerance [5,8] -Greater belowground competitive ability[3]	-High foraging capacity	- Thicker roots - Longer life span[9] -Associated with higher substrate penetrability when dry soil becomes compacted[10]	-Durable and long lasting roots
Root length ratio (RLR) SRL x RMF	-Low root tissue density[1] -High foraging capacity of energetically inexpensive tissue[1] - Short life-span[1] - Higher root surface area and drought tolerance[8] -High growth rate[1] - Fast resource capture[1] -Positively correlated to nitrogen uptake[1]	- Short lived roots with fast resource capture	-Slower root turnover with less nutrient needs[11] -Lower resource capture	-Long lasting and low resource demand roots

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Table 2. *Elymus elymoides* accessions and cheatgrass seed source information.

Species	Source Population	Code	Elevation	Field Coordinates ºN	ºW	Seed Source
Bromus tectorum	DeBeque, CO	BRTE	1509	39.3349	-108.2151	Wild collected
Elymus elymoides	Duchesne, UT	Commercial	1682	38.4832	-107.8758	Commercially available
Elymus elymoides	Montrose, CO	Montrose	1770	40.1669	-110.4028	Wild collected
Elymus elymoides	Ashley Nat'l Forest, UT	Ashley	2194	39.9831	-110.3374	Wild collected
Elymus elymoides	Dixie Nat'l Forest, UT	Dixie	2623	37.6554	-112.6694	Wild collected
Elymus elymoides	Dixie Nat'l Forest, UT	Dixie II	2900	38.0417	-111.3247	Wild collected
Elymus elymoides	Manti Nat'l Forest, UT	Manti	1930	38.9146	-111.2653	Wild collected
Elymus elymoides	Manti Nat'l Forest, UT	Manti II	2423	37.8695	-109.6920	Wild collected
Elymus elymoides	Fishlake Nat'l Forest, UT	Fishlake I	2506	38.6406	-111.4316	Wild collected
Elymus elymoides	Fishlake Nat'l Forest, UT	Fishlake II	2403	38.7730	-111.4834	Wild collected
Elymus elymoides	Fishlake Nat'l Forest, UT	Fishlake	1814	37.7378	-111.6281	Wild collected
Elymus elymoides	UP, CO	UP	1782	38.4114	-111.6339	Wild collected
Elymus elymoides	UP, CO	UP II	2433	38.3679	-108.0235	Wild collected

Table 3. Table summarizing plant traits from common garden experiment. Mean final root length, days to germinate, germination response, and lateral root number of 21 day-old seedlings.

Accession	Root Length (mm)	Germination Response	Days to germinate	Lateral Root Number	
				High	Low
Ashley	85.41	52.5%	23.2	12.3	15.9
BRTE	57.54	100%	8.0	9.5	13.1
Commercial	67.43	90%	22.1	5.1	2.5
Dixie	80.58	85%	16.6	3.3	5
Dixie II	54.57	70%	28.3	6	8.8
Fishlake	93.73	97.5%	15.7	3.3	6.4
Fishlake I	N/A	20.5%	29.2	5.4	6.9
Fishlake II	N/A	62.5%	N/A	N/A	N/A
Manti	95.93	85%	13.2	5.6	6.9
Manti II	76.96	87.5%	19.6	3.4	8.9
Montrose	74.1	97.5%	19.6	3.4	3.4
UP	N/A	27.5%	27.6	N/A	N/A
UP II	N/A	32.5%	25	N/A	N/A

N/A indicates where the sample size was too small due to low germination response and/or high mortality.

Table 4. Model results from traits measured from common garden experiment (a) Linear model (b) Linear Mixed effects model (c) GLM.

Response variable	Nutrient	Accession	Nutrient x Accession	DF	F
(a)					
Root mass	n.s.	n.s.	n.s.	256	---
Shoot mass	***	n.s.	n.s.	212	67.9
(b)					
Root length	n.s.	***	n.s.	213	4.78
Root elongation rate	n.s.	***	n.s.	318	6.3
(c)					
Main root axes	n.s.	n.s.	n.s.	213	---
Lateral Roots	*	***	n.s.	204	20.6
Days to germinate	n.s.	***	n.s.	316	37.7

Significance codes: *** $p<0.0001$, ** $p<0.001$, * $p<0.05$, + (marginally significant) $p=0.05$, ns $p>0.05$

Table 5. Model results of *Elymus elymoides* plant traits from Water Stress Experiment of 105 day-old juveniles from two Accessions. (a) Linear mixed effects model (b) GLM and (c) Survivorship analysis.

Response variable	Water stress	Accession	Water stress x Accession	F
(a)				
Root mass	**	n.s.	ns	---
Shoot mass	+	***	ns	29.6
Root length	n.s.	n.s.	ns	---
Shoot height	n.s.	n.s.	ns	---
RMF	n.s.	***	ns	52.5
RLR	*	***	ns	16.5
SRL	**	n.s.	ns	7.1
(b)				
Leaf number	*	n.s.	ns	28.4
(c)				
Survivorship	n.s.	*	n.s.	---

Significance codes: *** p<0.0001, ** p<0.001, * p<0.05,
+ (marginally significant) p=0.05, ns p>0.05